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(54) NOVEL HEMOPOIETIN RECEPTOR PROTEINS

(57) The present invention provides novel hemopoietin receptor proteins (proteins comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 19, or 21), proteins comprising a modified amino acid sequence of the amino acid sequence of the above protein in which one or more amino acids have been deleted, added, and/or replaced with another amino acid, genes encoding these proteins, methods of producing the proteins, as well as uses of these proteins and genes.

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DescriptionTechnical Field

5 [0001] The present invention relates to novel hemopoietin receptor proteins, the encoding genes, and methods of production and uses thereof.

Background Art

10 [0002] A large number of cytokines are known as humoral factors that are involved in the proliferation/differentiation of various cells, or activation of differentiated mature cells, and also cell death. These cytokines have their own specific receptors, which are categorized into several families based on their structural similarities (Hilton D.J., in "Guidebook to Cytokines and Their Receptors" edited by Nicola N.A. (A Sambrook & Tooze Publication at Oxford University Press), 1994, p8-16).

15 [0003] Compared to similarities between receptors, primary-structure homology is quite low between cytokines, and a significant amino acid homology cannot be seen even among cytokine members that belong to the same receptor family. This explains the functional specificity of each cytokine, as well as similarities of cellular reactions induced by each cytokine.

20 [0004] Representative examples of the above-mentioned receptor families are the tyrosine kinase receptor family, hemopoietin receptor family, tumor necrosis factor (TNF) receptor family, and transforming growth factor β (TGF β) receptor family. Different signal transduction pathways have been reported to be involved in each of these families. Among these receptor families, many receptors of especially the hemopoietin receptor family are expressed in blood cells and immunocytes, and their ligands, cytokines, are often termed as hemopoietic factors or interleukins. Some of these hemopoietic factors or interleukins exist within blood and are thought to be involved in a systemic humoral regulation of hemopoietic or immune functions.

25 [0005] This contrasts with the belief that cytokines belonging to other families are often involved in only topical regulations. Some of these hemopoietins can be taken as hormone-like factors, and conversely, representative peptide hormones such as the growth hormone, prolactin, or leptin receptors also belong to the hemopoietin receptor family. Because of these hormone-like systemic regulatory features, it is anticipated that hemopoietin administration would be applied in the treatment of various diseases.

30 [0006] Among the large number of cytokines, those that are actually being clinically applied are, erythropoietin, G-CSF, GM-CSF, and IL-2. Combined with IL-11, LIF, and IL-12 that are being considered for clinical trials, and the above-mentioned peptide hormones such as growth hormone and prolactin, it can be envisaged that by searching among the above-mentioned various receptor families for a novel cytokine that binds to hemopoietin receptors, it is possible to find 35 a cytokine that can be clinically applied with a higher efficiency.

35 [0007] As mentioned above, cytokine receptors have structural similarities between the family members. Using these similarities, many investigations are being carried out aiming at finding novel receptors. Regarding the tyrosine kinase receptor especially, many receptors have already been cloned using its highly conserved sequence at the catalytic site (Matthews W. et al., Cell, 1991, 65 (7) p1143-52). Compared to this, hemopoietin receptors do not have a tyrosine kinase-like enzyme activity domain in their cytoplasmic regions, and their signal transductions are known to be mediated through associations with other tyrosine kinase proteins existing freely in the cytoplasm.

40 [0008] Though the binding site on receptors associating with these cytoplasmic tyrosine kinases (JAK kinases) is conserved between family members, the homology is not very high (Murakami M. et al., Proc. Natl. Acad. Sci. USA, 1991, 88, 11349-11353). On one hand, the sequence that characterizes these hemopoietin receptors most well exists 45 in the extracellular region, and especially the five amino acid Trp-Ser-Xaa-Trp-Ser (where Xaa is an arbitrary amino acid) motif is conserved in almost all of the hemopoietin receptors. Therefore, novel receptors are expected to be obtained by searching novel family members using this sequence. In fact, this approach has already identified the IL-11 receptor (Robb, L. et al., J. Biol. Chem., 1996, 271 (23) 13754-13761), leptin receptor (Gainsford T. et al., Proc. Natl. Acad. Sci. USA, 1996, 93 (25) p14564-8) and the IL-13 receptor (Hilton D.J. et al., Proc. Natl. Acad. Sci. USA, 1996, 93 50 (1) p497-501).

Disclosure of the Invention

55 [0009] The present invention provides a novel hemopoietin receptor protein, and the encoding DNA. The present invention also provides, a vector into which the DNA has been inserted, a transformant harboring the DNA, and a method of producing a recombinant protein using the transformant. It also provides a method of screening a compound that binds to the protein.

[0010] Until now, the inventors have been trying to search for a novel receptor using an oligonucleotide encoding

the Trp-Ser-Xaa-Trp-Ser motif as a probe by plaque hybridization, RT-PCR method, and so on. However, because of reasons such as the oligonucleotide tggag (t/c) nnntggag (t/c) (where n is an arbitrary nucleotide) that encodes the motif being short having just 15 nucleotides, and the g/c being high, it was extremely difficult to strictly select only those in which the 15 nucleotides have completely hybridized under the usual hybridization conditions.

5 [0011] Also, a similar sequence is contained within cDNA encoding proteins other than hemopoietin receptors, starting with various collagens that are thought to be widely distributed and also have high expression amounts, which makes the screening by the above-mentioned plaque hybridization and RT-PCR highly inefficient.

[0012] To solve these problems, and to estimate how many different hemopoietic receptor genes actually exist on the human genome, the inventors computer-searched sequences that completely coincided with each probe using all 10 capable oligonucleotide sequences encoding the above-mentioned Trp-Ser-Xaa-Trp-Ser motif as probes.

[0013] Next, among the clones identified by the above search, the nucleotide sequence around the probe sequence of human genome-derived clones (cosmid, BAC, PAC) was converted to the amino acid sequence and compared with the amino acid sequence of known hemopoietin receptors to select human genes thought to encode hemopoietin receptor family members.

15 [0014] From the above search, two clones thought to be hemopoietin receptor genes were identified. One of these was the known GM-CSF β receptor gene (derived from the 22q12.3-13.2 region of chromosome no. 22), and the other (BAC clone AC002303 derived from the 16p12 region of chromosome no. 16) was presumed to encode a novel hemopoietin receptor protein, and this human gene was named "NR8."

[0015] Next, the cDNA thought to encode NR8 was found within the human fetal liver cell cDNA library by RT-PCR 20 using a specific primer designed based on the obtained nucleotide sequence. Furthermore, using this cDNA library as the template, the full-length cDNA NR8 α encoding a transmembrane receptor comprising 361 amino acids was ultimately obtained by 5'-RACE method and 3'-RACE method.

[0016] In the primary structure of NR8 α , a cysteine residue and a proline rich motif conserved between other family 25 members, were well conserved in the extracellular region, and in the intracellular region, the Box 1 motif thought to be involved in signal transduction was well conserved, and therefore, NR8 α was thought to be a typical hemopoietin receptor.

[0017] Furthermore, the inventors revealed the presence of two genes named NR8 β and NR8 γ as selective splicing products of NR8 α .

[0018] The inventors next attempted the isolation of the mouse gene corresponding to NR8 gene. First, using an 30 oligonucleotide primer designed within human NR8 cDNA sequence and a mouse brain cDNA library as the template, xenogeneic cross PCR cloning was done to isolate the mouse partial nucleotide sequence of the above receptor. Furthermore, based on the obtained partial sequence, an oligonucleotide primer was designed, and using this, the inventors succeeded in isolating the full-length ORF of the mouse homologous gene corresponding to NR8 by the 5'-RACE method and 3'-RACE method. As a result of determining the whole nucleotide sequence of the obtained cDNA clone, 35 alike NR8, the presence of mouse NR8 γ encoding a transmembrane receptor protein comprising 538 amino acids, and mouse NR8 β encoding a secretory, soluble receptor-like protein comprising 144 amino acids were confirmed by the difference of transcripts derived from the splice variant. When the amino acid sequences encoded by these receptor genes were compared between human and mouse, a high homology of 98.9% was observed for NR8 γ , and on the other hand, a homology of 97.2% was seen for NR8 β as well. Furthermore, the inventors succeeded in isolating the objective 40 positive clones by plaque screening against a mouse genomic DNA library using the obtained mouse NR8 β cDNA fragment as the probe.

[0019] Therefore, the present invention provides:

(1) a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ 45 ID NO: 1, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1;

(2) a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ 50 ID NO: 3, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3;

(3) a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ 55 ID NO: 5, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5;

(4) a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7;

(5) a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19;

(6) a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added, and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21;

(7) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 2, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1;

(8) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 4, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3;

(9) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 6, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5;

(10) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 8, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7;

(11) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 20, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19;

(12) a protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 22, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21;

(13) a fusion protein comprising the protein of any one of (1) to (12) and another peptide or polypeptide;

(14) a DNA encoding the protein of any one of (1) to (13);

(15) a vector comprising the DNA of (14);

(16) a transformant harboring the DNA of (14) in an expressible manner;

(17) a method of producing the protein of any one of (1) to (13), comprising the step of culturing the transformant of (16);

(18) a method of screening a compound that binds to the protein of any one of (1) to (13) comprising the steps of,

- (a) contacting a test sample with the protein of any one of (1) to (13), and
- (b) selecting a compound that comprises an activity to bind to the protein of any one of (1) to (13);

(19) an antibody that specifically binds to the protein of any one of (1) to (12);

(20) a method of detecting or measuring the protein of any one of (1) to (13) comprising the steps of contacting a test sample presumed to contain said protein with the antibody of (19), and detecting or measuring the formation of the immune complex between the antibody and the protein; and

(21) a DNA specifically hybridizing to a DNA comprising the nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, 20, and 22 to 27, and comprising at least 15 nucleotides.

[0020] The present invention relates to the novel hemopoietin receptor "NR8." 5'-RACE and 3'-RACE analyses, NR8 genome sequence analysis, and plaque screening analysis revealed the presence of NR8 α , NR8 β , and NR8 γ . The structures of these NR8 genes are shown in Fig. 13. Among the NR8 genes, NR8 β is an alternative splicing product lacking the 5th exon, and can encode two different proteins, a soluble protein in which the CDS ends with a stop codon on the 6th exon that results from a frame shift following direct coupling to the 4th exon, and a membrane-bound protein lacking the signal sequence starting from the ATG upon the 4th exon.

[0021] Since the soluble protein comprises the same sequence as NR8 α up to the 4th exon, it may function as a

soluble receptor. On the other hand, NR8 γ encodes a protein containing a 177 amino acid insertion derived from the NR8 9th intron close to the C terminus of the NR8 α as a result of selective splicing.

[0022] Both NR8 α and NR8 γ encode transmembrane-type hemopoietin receptors. Among the sequences conserved between other hemopoietin receptors that are thought to be involved in signal transduction, a motif resembling Box 1 exists in the intracellular domain of NR8 α and NR8 γ adjacent to the cell membrane. Though low in the degree of conservation, a sequence that is similar to Box 2 also exists, and therefore, NR8 is thought to be a type of receptor in which the signal is transduced by a homodimer.

[0023] The amino acid sequences of the NR8 proteins included in the proteins of the present invention are shown in SEQ ID NO: 1 (NR8 α), SEQ ID NO: 3 (soluble NR8 β), SEQ ID NO: 5 (membrane-bound NR8 β), and SEQ ID NO: 7 (NR8 γ), and the nucleotide sequences of cDNA encoding these proteins are shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8, respectively.

[0024] Northern blot analysis for the spleen, thymus, peripheral leucocytes, and lung showed two to three bands in the 5kb and 3 to 4kb regions. Similar sized bands were observed for cell lines HL60 and Raji also, but no expression was seen for other tumor cell lines (HeLa, SW480, A549, G361) and leukemia cell lines (K562, MOLT4).

[0025] The above results suggest that NR8 is specifically expressed on hemopoietic cell lines, especially on granulocytic lines, and B cell lines.

[0026] The above NR8 protein is expected to be applied in medicine. NR8 is expressed in fetal liver, spleen, thymus, and some leukemic cell lines, suggesting the possibility that it might be a receptor of an unknown hemopoietic factor. Therefore, NR8 protein would be a useful material for obtaining this unknown hemopoietic factor.

[0027] Furthermore, it is possible that NR8 is specifically expressed in limited cell populations within these hemopoietic tissues, and therefore, anti NR8 antibody may be useful as a means of separating these cell populations. Thus separated cell populations can be applied for cell transplant therapy. Anti NR8 antibody is also expected to be applied for the diagnosis and treatment of leukemic diseases represented by leukemia.

[0028] On the other hand, the soluble protein including the extracellular domain of NR8 protein, or NR8 β , a splicing variant of NR8, may be applied as a decoy-type receptor that is an inhibitor of the NR8 ligand, and is anticipated to be applied in the treatment of diseases in which NR8 is involved, starting with leukemia.

[0029] The inventors also isolated mouse NR8 cDNA corresponding to the human-derived NR8 cDNA above-mentioned, by using the xenogeneic cross PCR cloning method. The amino acid sequences of the proteins named mouse NR8, which are included in the protein of the present invention are shown in SEQ ID NO: 19 (soluble mouse NR8 β) and SEQ ID NO: 21 (mouse NR8 γ), and the nucleotide sequences of the cDNA encoding these proteins are shown in SEQ ID NO: 20 and SEQ ID NO: 22, respectively.

[0030] As a result of structural analysis of the obtained mouse cDNA clones, alike human-derived NR8, the presence of mouse NR8 γ encoding a transmembrane receptor protein comprising 538 amino acids and mouse NR8 β encoding a secretory soluble receptor-like protein comprising 144 amino acids which were confirmed by the difference of transcripts derived the splice variant, was confirmed. When the amino acid sequences encoded by these receptor genes were compared between human and mouse, a high homology of 98.9% was observed for NR8 γ , while a homology of 97.2% was seen for NR8 β as well.

[0031] Northern blot analysis and RT-PCR analysis showed that although there were deviations in expression levels, mouse NR8 gene expression was seen in all organs analyzed, and seemed to be widely distributed compared to human NR8, for which a strong expression was seen only in immunocompetent and hemopoietic tissues. This also suggests the possibility that molecular functions of mouse NR8 may span a broad range of physiological regulatory mechanisms of the body.

[0032] The present invention also encompasses a protein that is functionally equivalent to the above-mentioned human or mouse NR8 protein. Herein "functionally equivalent" means having an equivalent biological activity to the above-mentioned NR8 proteins. Hemopoietic factor receptor protein activity can be given as an example of a biological activity. Such proteins can be obtained by the method of introducing a mutation to the amino acid sequence of a protein. For example, site-specific mutagenesis using a synthetic oligonucleotide primer, can be used to introduce a desired mutation into the amino acid sequence of a protein (Kramer, W. and Fritz, H.J., Methods in Enzymol., 1987, 154, 350-367). This could also be done by a PCR-mediated site-specific mutagenesis system (GIBCO-BRL). Using these methods, the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 19, or SEQ ID NO: 21 can be modified to obtain a protein functionally equivalent to the NR8 protein, in which one or more amino acids in the amino acid sequence of the protein have been deleted, added, and/or substituted by another amino acid without affecting the biological activity of the protein.

[0033] As a protein functionally equivalent to the NR8 protein of the invention, the following are given: one in which one or two or more, preferably, two to 30, more preferably, two to ten amino acids are deleted in any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 19, or SEQ ID NO: 21; one in which one or two or more, preferably, two to 30, more preferably, two to ten amino acids have been added into any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7; or one in which

one or two or more, preferably, two to 30, more preferably, two to ten amino acids have been substituted with other amino acids in any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7.

5 [0034] It is already known that a protein comprising a modified amino acid sequence of a certain amino acid sequence in which one or more amino acid residues have been deleted, added, and/or substituted with another amino acid, still maintains its biological activity (Mark, D. F. et al., Proc. Natl. Acad. Sci. USA, 1984, 81, 5662-5666; Zoller, M. J. & Smith, M., Nucleic Acids Research, 1982, 10, 6487-6500; Wang, A. et al., Science, 224, 1431-1433; Dalbadie-McFarland, G. et al., Proc. Natl. Acad. Sci. USA, 1982, 79, 6409-6413).

10 [0035] For example, a fusion protein can be given as a protein in which one or more amino acid residues have been added to the NR8 protein of the present invention. A fusion protein is made by fusing the NR8 protein of the present invention with another peptide or protein and is encompassed in the present invention. A fusion protein can be prepared by ligating DNA encoding the NR8 protein of the present invention with DNA encoding another peptide or protein so as the frames match, introducing this into an expression vector, and expressing the fusion gene in a host. Methods commonly known can be used for preparing such a fusion gene. There is no restriction as to the other peptide or protein that is fused to the protein of this invention.

15 [0036] For example, FLAG (Hopp, T.P. et al., Biotechnology, 1988, 6, 1204-1210), 6x His constituting six histidine (His) residues, 10x His, Influenza agglutinin (HA), human c-myc fragment, VSV-GP fragment, p18HIV fragment, T7-tag, HSV-tag, E-tag, SV40T antigen fragment, Ick tag, α -tubulin fragment, B-tag, Protein C fragment, and such well-known peptides can be used. Examples of proteins are, glutathione-S-transferase (GST), Influenza agglutinin (HA), immunoglobulin constant region, β -galactosidase, maltose-binding protein (MBP), etc. Commercially available DNAs encoding these may also be used to prepare fusion proteins.

20 [0037] The protein of the invention can also be encoded by a DNA that hybridizes under stringent conditions to a DNA comprising any one of the nucleotide sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 20, and SEQ ID NO: 22 to 27. Such a protein also includes a protein functionally equivalent to the above-mentioned NR8 protein. Stringent conditions can be suitably selected by one skilled in the art, and for example, low-stringent conditions can be given. Low-stringent conditions are, for example, 42°C, 2x SSC, and 0.1% SDS, and preferably, 50°C, 2x SSC, and 0.1% SDS. More preferable are highly stringent conditions, for example, 65°C, 2x SSC, and 0.1% SDS. Under these conditions, the higher the temperature is raised, the higher the homology of the obtained DNA will be.

25 [0038] The present invention also includes a protein that is functionally equivalent to the above NR8 protein, which has also a homology with a protein comprising any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 19, or SEQ ID NO: 21. A protein having a homology means, a protein having at least 70%, preferably at least 80%, more preferably at least 90%, even more preferably, at least 95% homology to any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 7. The homology of a protein can be determined by the algorithm in "Wilbur, W.J. and Lipman, D.J. Proc. Natl. Acad. Sci. USA, 1983, 80, 726-730."

30 [0039] In the protein of the invention, the amino acid sequence, molecular weight, isoelectric point, the presence or absence of the sugar chain, and its form differ according to the producing cells, host, or purification method described below. However, as long as the obtained, protein comprises a hemopoietic factor receptor protein activity, it is included in the present invention.

35 [0040] For example, if the protein of the present invention is expressed in prokaryotic cells such as *E. coli*, a methionine residue is added at the N-terminus of the amino acid sequence of the expressed protein. If the protein of the present invention is expressed in eukaryotic cells such as mammalian cells, the N-terminal signal sequence is removed. The protein of the present invention includes these proteins.

40 [0041] For example, as a result of analyzing the protein of the invention based on the method in "Von Heijne, G., Nucleic Acids Research, 1986, 14, 4683-4690," it was presumed that the signal sequence is from the 1st Met to the 19th Gly in the amino acid sequence of SEQ ID NO: 1. Therefore, the present invention encompasses a protein comprising the sequence from the 20th Cys to 361st Ser in the amino acid sequence of SEQ ID NO: 1.

45 [0042] To produce the protein of the invention, the obtained DNA is incorporated into an expression vector in a manner that the DNA is expressible under the regulation of an expression regulatory region, for example, an enhancer or promoter. Next, host cells are transformed by this expression vector to express the protein.

50 [0043] Specifically, the protein can be produced as follows. When mammalian cells are used, DNA comprising a commonly used useful promoter/enhancer, DNA encoding the protein of the invention, and the poly A signal that is functionally bound to the 3' side downstream of the protein-encoding DNA, or a vector containing it, is constructed. For example, as the promoter/enhancer, human cytomegalovirus immediate early promoter/enhancer can be given.

55 [0044] Also, as other promoters/enhancers that can be used for protein expression, viral promoters/enhancers of retroviruses, polyomaviruses, adenoviruses, simian virus 40 (SV40), and such, and promoters/enhancers derived from mammalian cells, such as that of human elongation factor 1 α (HEF1 α) can be used.

[0045] For example, a protein can be easily expressed by following the method of Mulligan et al. (Nature, 1979, 277,

108) when using the SV40 promoter/enhancer, and the method of Mizushima et al. (Nucleic Acids Res., 1990, 18, 5322) when using the HEF1 α promoter/enhancer.

[0046] When using *E. coli*, well-used useful promotors, the signal sequence for polypeptide secretion, and genes to be expressed, may be functionally bound to express the desired gene. For example, lacZ promoter and araB promoter may be used as promotors. When using the lacZ promoter, the method of Ward et al. (Nature, 1998, 341, 544-546; FASEB J., 1992, 6, 2422-2427), and when using the araB promoter, the method of Better et al. (Science, 1988, 240, 1041-1043) may be followed.

[0047] When producing the protein into the periplasm of *E. coli*, the pelB (Lei, S. P. et al., J. Bacteriol., 1987, 169, 4379) signal sequence may be used as a protein secretion signal.

[0048] A replication origin derived from SV40, polyomavirus, adenovirus, bovine papillomavirus (BPV), and such may be used. To amplify gene copies in host cell lines, the expression vector may include an aminoglycoside transferase (APH) gene, thymidine kinase (TK) gene, *E. coli* xanthine guanine phosphoribosyl transferase (Ecogpt) gene, dihydrofolate reductase (dhfr) gene, and such as a selective marker.

[0049] The expression vector used to produce the protein of the invention may be any, as long as it's an expression vector that is suitably used for the present invention. Mammalian expression vectors, for example, pEF and pCDM8; insect-derived expression vectors, for example, pBacPAK8; plant-derived expression vectors, for example, pMH1 and pMH2; animal virus-derived expression vectors, for example, pHSV, pMV, and pAdexLcw; retrovirus-derived expression vectors, for example, pZlpneo; yeast-derived expression vectors, for example, pNV11 and SP-Q01; *Bacillus subtilis*-derived expression vectors, for example, pPL608 and pKTH50; *E. coli*-derived expression vectors, for example, pQE, pGEAPP, pGEMEAPP, and pMALp2 can be given as expression vectors of this invention.

[0050] Not only vectors that produce the protein of the invention *in vivo* and *in vitro*, but also those that are used for gene therapy of mammals, for example humans, are also included as vectors of the present invention.

[0051] When introducing the expression vector of the present invention constructed above into a host cell, well-known methods, for example the calcium phosphate method (Virology, 1973, 52, 456-467), electroporation (EMBO J., 1982, 1, 841-845), and such may be used.

[0052] In the present invention, an arbitrary production system may be used to produce the protein. *In vitro* and *in vivo* production systems are known as production systems for producing proteins. Production systems using eukaryotic cells and prokaryotic cells may be used as *in vitro* production systems.

[0053] When using eukaryotic cells, production systems using, for example, animal cells, plant cells, and fungal cells are known. As animal cells used, for example, mammalian cells such as CHO (J. Exp. Med., 1995, 108, 945), COS, myeloma, baby hamster kidney (BHK), HeLa, or Vero, amphibian cells such as *Xenopus* oocytes (Valle, et al., Nature, 1981, 291, 358-340), insect cells such as sf9, sf21, or Tn5, are known. As CHO cells, especially DHFR gene-deficient CHO cell, dhfr-CHO (Proc. Natl. Acad. Sci. USA, 1980, 77, 4216-4220), and CHO K-1 (Proc. Natl. Acad. Sci. USA, 1968, 60, 1275) can be suitably used.

[0054] *Nicotiana tabacum*-derived cells are well known as plant cells, and these can be callus cultured. As fungal cells, yeasts such as the *Saccharomyces* genus, for example, *Saccharomyces cerevisiae*, filamentous bacteria such as the *Aspergillus* genus, for example, *Aspergillus niger* are known.

[0055] Bacterial cells may be used as prokaryotic production systems. As bacterial cells, *E. coli* and *Bacillus subtilis* are known.

[0056] Proteins can be obtained by transforming these cells with the objective DNA, and culturing the transformed cells *in vitro* according to well-known methods. For example, DMEM, MEM, RPMI1640, and IMDM can be used as culture media. At that instance, fetal calf serum (FCS) and such serum-supplements may be added in the above media, or a serum-free culture medium may be used. The pH is preferably about 6 to 8. Culture is usually done at about 30°C to 40°C, for about 15 to 200 hr, and medium changes, aeration, and stirring are done as necessary.

[0057] On the other hand, production systems using animals and plants may be given as *in vivo* production systems. The objective gene is introduced into the plant or animal, and the protein is produced within the plant or animal, and recovered. "Host" as used in the present invention encompasses such animals and plants as well.

[0058] When using animals, mammalian and insect production systems can be used. As mammals, goats, pigs, sheep, mice, and cattle may be used (Vicki Glaser, SPECTRUM Biotechnology Applications, 1993). Transgenic animals may also be used when using mammals.

[0059] For example, the objective DNA is inserted within a gene encoding a protein produced intrinsically into milk, such as goat β casein, to prepare a fusion gene. The DNA fragment containing the fusion gene is injected into a goat's embryo, and this embryo is implanted in a female goat. The protein is collected from the milk of the transgenic goats produced from the goat that received the embryo, and descendants thereof. To increase the amount of protein-containing milk produced from the transgenic goat, a suitable hormone/hormones may be given to the transgenic goats (Ebert, K.M. et al., Bio/Technology, 1994, 12, 699-702).

[0060] Silk worms may be used as insects. When using the silk worm, it is infected with a baculovirus to which the objective DNA has been inserted, and the desired protein is obtained from the body fluids of the silk worm (Susumu, M.

et al., *Nature*, 1985, 315, 592-594).

[0061] When using plants, for example, tobacco can be used. In the case of tobacco, the objective DNA is inserted into a plant expression vector, for example pMON 530, and this vector is introduced into a bacterium such as *Agrobacterium tumefaciens*. This bacterium is infected to tobacco, for example *Nicotiana tabacum*, to obtain the desired 5 polypeptide from tobacco leaves (Julian, K.-C. Ma et al., *Eur. J. Immunol.*, 1994, 24, 131-138).

[0062] The thus-obtained protein of the invention is isolated from within and without cells, or from hosts, and can be purified as a substantially pure homogenous protein. The separation and purification of the protein is not limited to any specific method and can be done using ordinary separation and purification methods used to purify proteins. For example, chromatography, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, recrystallization, and such may be suitably selected, or combined to separate/purify the protein.

[0063] As chromatographies, for example, affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration, reversed-phase chromatography, adsorption chromatography, and such can be exemplified (Strategies for Protein Purification and Characterization: A Laboratory Course Manual. Ed Daniel R. Marshak et al., 15 Cold Spring Harbor Laboratory Press, 1996). These chromatographies can be done by liquid chromatography such as HPLC, FPLC, and the like. The present invention encompasses proteins highly purified by using such purification methods.

[0064] Proteins can be arbitrarily modified, or peptides may be partially excised by treating the proteins with appropriate modification enzymes prior to or after the purification. Trypsin, chymotrypsin, lysyl endopeptidase, protein kinase, 20 glucosidase, and such are used as protein modification enzymes.

[0065] The present invention includes a partial peptide comprising the active center of a protein comprising any one of the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 19, and SEQ ID NO: 21. A partial peptide of the protein of the present invention is, for example, a partial peptide of the molecules of the protein, which contains one or more regions of the hydrophilic region and hydrophobic region presumed by hydrophobicity plot analysis. These partial peptides may contain the whole hydrophilic region or a part of it, and may contain the whole hydrophobic region or a part of it. For example, soluble proteins and proteins comprising extracellular regions of the protein of the invention, are also encompassed in the invention.

[0066] The partial peptides of the protein of the invention may be produced by genetic engineering techniques, well-known peptide synthesizing methods, or by excising the protein of the invention by a suitable peptidase. As peptide synthesizing methods, the solid-phase synthesizing method, and the liquid-phase synthesizing method may be used.

[0067] The present invention also relates to a DNA encoding the protein of the invention. A cDNA encoding the protein of the invention may be obtained by, for example, screening a human cDNA library using the probe described herein.

[0068] Using the obtained cDNA or cDNA fragment as a probe, cDNA can also be obtained from other cells, tissues, organs, or species by further screening cDNA libraries. cDNA libraries may be prepared by, for example, the method of Sambrook, J. et al., *Molecular Cloning*, Cold Spring Harbor Laboratory Press (1989), or commercially available cDNA libraries may be used.

[0069] By determining the nucleotide sequence of the obtained cDNA, the translation region encoded by it can be determined, and the amino acid sequence of the protein of the present invention can be obtained. Furthermore, 40 genomic DNA can be isolated by screening the genomic DNA library using the obtained cDNA as a probe.

[0070] Specifically, this can be done as follows. First, mRNA is isolated from cells, tissues, and organs expressing the protein of the invention. For this mRNA isolation, whole RNA is prepared using well-known methods, for example, guanidine ultracentrifugation method (Chirgwin, J.M. et al., *Biochemistry*, 1979, 18, 5294-5299), the AGPC method (Chomczynski, P. and Sacchi, N., *Anal. Biochem.*, 1987, 162, 156-159), and such, and purified using the mRNA Purification Kit (Pharmacia), etc. mRNA may be directly prepared using the QuickPrep mRNA Purification Kit (Pharmacia).

[0071] cDNA is synthesized using reverse transcriptase from the obtained mRNA. cDNA can be synthesized using the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (SEIKAGAKU CORPORATION), etc. Also, cDNA synthesis and amplification may also be done using the probe described herein by following the 5'-RACE method (Frohman, M.A. et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1988, 85, 8998-9002; Belyavsky, A. et al., *Nucleic Acids Res.*, 1989, 50 17, 2919-2932) using the polymerase chain reaction (PCR) and the 5'-Ampli FINDER RACE KIT (Clontech).

[0072] The objective DNA fragment is prepared from the obtained PCR product and ligated with vector DNA. Thus, a recombination vector is created, introduced into *E.coli*, etc. and colonies are selected to prepare the desired recombination vector. The nucleotide sequence of the objective DNA may be verified by known methods, for example, the dideoxy nucleotide chain termination method.

[0073] In the DNA of the invention, a sequence with a higher expression efficiency can be designed by considering the codon usage frequency of hosts used for the expression (Grantham, R. et al., *Nucleic Acids Research*, 1981, 9, p43-p74). The DNA of the invention may also be modified using commercially available kits and known methods. For example, digestion by restriction enzymes, insertion of synthetic oligonucleotides and suitable DNA fragments, addition

of linkers, insertion of a start codon (ATG) and/or stop codon (ATT, TGA, or TAG), and such can be given.

[0074] The DNA of the present invention encompasses DNA comprising the nucleotide sequence from the 441st nucleotide A to the 1523rd nucleotide C in the nucleotide sequence of SEQ ID NO: 2, DNA comprising the nucleotide sequence from the 441st nucleotide A to the 872nd nucleotide A in the nucleotide sequence of SEQ ID NO: 4, DNA comprising the nucleotide sequence from the 659th nucleotide A to the 1368th nucleotide C in the nucleotide sequence of SEQ ID NO: 6, DNA comprising the nucleotide sequence from the 441st nucleotide A to the 2054th nucleotide C in the nucleotide sequence of SEQ ID NO: 8, DNA comprising the nucleotide sequence from the 439th nucleotide A to the 870th nucleotide A in the nucleotide sequence of SEQ ID NO: 20, and DNA comprising the nucleotide sequence from the 439th nucleotide A to the 2052nd nucleotide C in the nucleotide sequence of SEQ ID NO: 22.

5 [0075] The DNA of the present invention encompasses DNA that hybridizes under stringent conditions to the DNA comprising any one of the nucleotide sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 20, and SEQ ID NO: 22 to 27, which also includes a DNA encoding a protein functionally equivalent to the NR8 protein.

10 [0076] Stringent conditions can be suitably selected by one skilled in the art, and for example, low-stringent conditions can be given. Low-stringent conditions are, for example, 42°C, 2x SSC, and 0.1% SDS, and preferably 50°C, 2x SSC, and 0.1% SDS. More preferable are highly stringent conditions, for example, 65°C, 2x SSC, and 0.1% SDS. Under these conditions, the higher the temperature is raised, the higher the homology of the obtained DNA will be. The above DNA is preferably natural DNA such as cDNA and chromosomal DNA.

15 [0077] As shown in Examples, the mRNA of the gene hybridizing to cDNA encoding the protein of the invention was distributed in various human tissues. Therefore, the above-mentioned natural DNA may be, for example, genomic DNA and cDNA derived from tissues in which the mRNA that hybridizes to the cDNA encoding the protein of the invention is detected in Examples. The DNA encoding the protein of the invention may be cDNA, genomic DNA, or synthetic DNA.

20 [0078] The protein of the invention is useful in screening a compound that binds to it. Namely, the protein of the invention is used in the screening method that comprises the steps of contacting a test sample expected to contain a compound that binds to the protein of the invention with the protein of the invention, and selecting the compound that comprises an activity to bind to the protein of the invention.

25 [0079] As methods for screening a compound that comprises an activity to bind to the protein of the invention, numerous methods usually used by those skilled in the art can be employed. The protein of the invention that is used for the screening of the invention may be a recombinant, natural, or partial peptide. A compound comprising an activity to bind to the protein of the invention may be a protein comprising a binding activity, or it may be a chemically synthesized compound having a binding activity.

30 [0080] As a test sample that is used in the screening method of the present invention, for example, peptides, purified or crudely purified proteins, non-peptide compounds, synthetic compounds, microbial fermentation products, extracts of marine organisms, plant extracts, cell extracts, animal tissue extracts, and such can be given. These test samples may be novel compounds, or well-known compounds.

35 [0081] A protein that binds to the protein of the invention can be screened by, for example, using the West-western blotting method (Skolnik, E.Y. et al., Cell, 1991, 65, 83-90). cDNA is isolated from cells, tissues, and organs presumed to express the protein binding to the protein of the invention, this is inserted into phage vectors, for example, λgt11, ZAPII, and such, to make a cDNA library, expressed on a plate containing a culture medium, the proteins expressed are fixed on a filter, this filter is reacted with the labeled, purified protein of the invention, and plaques expressing the protein bound to the protein of the invention are detected by the labels. As methods to label the protein of the invention, the method that uses the binding ability of avidin and biotin, the method of using an antibody that specifically binds to the protein of the invention or the peptide or polypeptide fused to the protein of the invention, the method of using radioisotopes, or fluorescence, and such can be given.

40 [0082] A ligand that binds specifically to the protein of the invention can be screened by, preparing a chimeric receptor by ligating the extracellular domain of the protein of the invention with the intracellular domain containing the transmembrane domain of a hemopoietin receptor protein comprising a known signal transduction ability, expressing this chimeric receptor on the cell surface of a suitable cell line, preferably, a cell line that can survive and proliferate under the presence of a suitable growth factor (a growth factor-dependent cell line), and culturing the cell line by adding a material that is expected to contain various growth factors, cytokines, or hemopoietic factors. This method uses the fact that the above-mentioned growth factor-dependent cell line survives and proliferates only when a ligand that specifically binds to the extracellular domain of the protein of the invention exists within the test material. Known hemopoietic receptors are, for example, the thrombopoietin receptor, erythropoietin receptor, G-CSF receptor, gp130, etc. However, the partner of the chimeric receptor used in the screening of the invention is not limited to these known hemopoietic receptors, and any may be used as long as a structure needed for the signal transduction activity is contained in the cytoplasmic domain. Growth factor-dependent cell lines are for example, IL-3-dependent cell lines starting with BaF3 and FDC-P1.

45 [0083] As a ligand that specifically binds to the protein of the invention, the possibility of not only soluble proteins,

but also cell membrane-binding proteins can be envisaged, though rare. In such cases, screening can be done by labelling the protein containing only the extracellular domain of the protein of the invention, or a fusion protein in which the partial sequence of another soluble protein has been added to this extracellular domain, and measuring the binding with cells expected to express the ligand. As examples of proteins containing only the extracellular domain of the protein of the invention, for example, a soluble receptor protein artificially made by inserting a stop codon to the N terminal side of the transmembrane domain, or NR8 β soluble protein may be used. On the other hand, as a fusion protein in which the partial sequence of another soluble protein has been added to the extracellular domain of the protein of the invention, for example, proteins prepared by adding immunoglobulin Fc site, FLAG peptide, etc. to the C terminus of the extracellular domain can be used. These soluble labeled proteins can be used in the detection in the above-described 10 West-western blotting method.

[0084] A protein that binds to the protein of the invention can be screened by using the two-hybrid system (Fields, S. and Sternglanz, R., Trends. Genet., 1994, 10, 286-292).

[0085] In the two-hybrid system, an expression vector containing DNA encoding the fusion protein between the protein of the invention and one subunit of a heterodimeric transcriptional regulatory factor, and an expression vector containing DNA made by ligating DNA encoding the other subunit of the heterodimeric transcriptional regulatory factor and a desired cDNA used as a test sample are introduced into cells and expressed. If the protein encoded by the cDNA binds with the protein of the invention and the transcriptional regulatory factor forms a heterodimer, a reporter gene constructed in the cell beforehand will be expressed. Therefore, a protein binding to the protein of the invention can be selected by detecting or measuring the expression level of the reporter gene.

[0086] Specifically, the DNA encoding the protein of the invention and the gene encoding the DNA binding domain of LexA are ligated so as the frames match to prepare an expression vector. Next, the desired cDNA and the gene encoding GAL4 transcription activation domain are ligated to prepare an expression vector.

[0087] Cells into which the HIS3 gene has been incorporated (the transcription of HIS3 gene is regulated by the promoter having a LexA binding motif) are transformed by the above two-hybrid system expression plasmids, and then 25 incubated on a histidine-free synthetic culture medium. Herein, cells only grow when a protein interaction is present. Thus, the increase in reporter gene expression can be examined by the growth rate of the transformant.

[0088] Other than the HIS3 gene, for example, the luciferase gene, plasminogen activator inhibitor type1 (PAI-1) gene, ADE2 gene, LacZ gene, CDC25H gene, and such can be used as reporter genes.

[0089] The two-hybrid system may be constructed according to the usual methods, or a commercially available kit 30 may be used. As commercially available two-hybrid system kits, the MATCHMARKER Two-Hybrid System, Mammalian MATCHMARKER Two-Hybrid Assay Kit (both by CLONTEC), HybriZAP Two-Hybrid Vector System (Stratagene), and CytoTrap two-hybrid system (Stratagene) can be given.

[0090] A protein binding to the protein of the invention can be screened by affinity chromatography. Namely, the protein of the invention is immobilized onto a carrier of an affinity column, and a test sample presumed to express a protein 35 binding to the protein of the invention is applied to the column. As this test sample, a cell culture supernatant, cell extract, cell lysate, and such may be used. After applying the test sample, the column is washed to obtain the protein binding to the protein of the invention.

[0091] The compound isolated by the screening method of the invention is a candidate drug for promoting or inhibiting the activity of the protein of the invention. The compound obtained by using the screening method of the invention 40 encompasses a compound resulting from modifying the compound having an activity to bind to the protein of the invention by adding, deleting, and/or replacing a part of the structure.

[0092] When using the compound obtained by the screening method of the invention as drugs for humans and other mammals such as, mice, rats, guinea pigs, rabbits, chicken, cats, dogs, sheep, pigs, cattle, monkeys, sacred baboons, and chimpanzees, the drug may be administered using ordinary means.

[0093] For example, according to the need, the drugs can be taken orally as sugar-coated tablets, capsules, elixirs, and microcapsules, or parenterally in the form of injections of sterile solutions or suspensions with water or any other pharmaceutically acceptable liquid. For example, the compounds comprising the activity to bind to the protein of the invention can be mixed with physiologically acceptable carriers, flavoring agents, excipients, vehicles, preservatives, stabilizers, and binders, in a unit dose form required for generally accepted drug implementation. The amount of active 50 ingredients in these preparations makes a suitable dosage within the indicated range acquirable.

[0094] Examples of additives that can be mixed to tablets and capsules are, binders such as gelatin, corn starch, tragacanth gum, and arabic gum; excipients such as crystalline cellulose; swelling agents such as cornstarch, gelatin, and alginic acid; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose, or saccharin; and flavoring agents such as peppermint, Gaultheria adenothrix oil, and cherry. When the unit dosage form is a capsule, a liquid carrier, such as oil, can also be included in the above additives. Sterile compositions for injections can be formulated following usual drug implementations using vehicles such as distilled water used for injections.

[0095] For example, physiological saline and isotonic liquids including glucose or other adjuvants, such as D-sorbitol, D-mannose, D-mannitol, and sodium chloride, can be used as aqueous solutions for injections. These can be used

in conjunction with suitable solubilizers, such as alcohol, specifically ethanol, polyalcohols such as propylene glycol and polyethylene glycol, non-ionic surfactants, such as Polysorbate 80 (TM) and HCO-50.

5 [0096] Sesame oil or soy-bean oil can be used as a oleaginous liquid and may be used in conjunction with benzyl benzoate or benzyl alcohol as a solubilizer; may be formulated with a buffer such as phosphate buffer and sodium acetate buffer; a pain-killer such as procaine hydrochloride; a stabilizer such as benzyl alcohol and phenol; and an antioxidant. The prepared injection is usually filled into a suitable ampule.

10 [0097] Although the dosage of the compound that has the activity to bind to the protein of the invention varies according to symptoms, the daily dose is generally about 0.1 to about 100 mg, preferably about 1.0 to about 50 mg, and more preferably about 1.0 to about 20 mg, when administered orally to an adult (body weight 60 kg).

15 [0098] When given parenterally, the dose differs according to the patient, target organ, symptoms, and method of administration, but the daily dose is usually about 0.01 to about 30 mg, preferably about 0.1 to about 20 mg and more preferably about 0.1 to about 10 mg for an adult (body weight 60 kg) when given as an intravenous injection. Also, in the case of other animals too, it is possible to administer an amount converted to 60 kg of body-weight.

19 [0099] The antibody of the present invention can be obtained as a monoclonal antibody or a polyclonal antibody using well-known methods.

[0100] The antibody that specifically binds to the protein of the invention can be prepared by using the protein of the invention as a sensitizing antigen for immunization according to usual immunizing methods, fusing the obtained immunized cells with known parent cells by ordinary cell fusion methods, and screening for antibody producing cells using the usual screening techniques.

20 [0101] Specifically, a monoclonal or polyclonal antibody that binds to the proteins of the invention may be prepared as follows.

25 [0102] For example, the protein of the invention that is used as a sensitizing antigen for obtaining the antibody is not restricted by the animal species from which it is derived, but is preferably a protein derived from mammals, for example, humans, mice, or rats, especially from humans. Proteins of human origin can be obtained by using the nucleotide sequence or amino acid sequence disclosed herein.

30 [0103] The protein that is used as a sensitizing antigen in the present invention can be a protein that comprises the biological activity of all the proteins described herein. Partial peptides of the proteins may also be used. As partial peptides of the proteins, for example, the amino (N) terminal fragment of the protein, and the carboxy (C) terminal fragment can be given. "Antibody" as used herein means an antibody that specifically reacts with the full-length or fragment of the protein.

35 [0104] A gene encoding the protein of the invention or a fragment thereof is inserted into a well-known expression vector, and after transforming the host cells described herein, the objective protein or a fragment thereof is obtained from within and without the host cell, or from the host using well-known methods, and this protein can be used as a sensitizing antigen. Also, cells expressing the protein, cell lysates, or chemically synthesized protein of the invention may be used as a sensitizing antigen.

40 [0105] The mammals that are immunized by the sensitizing antigen are not restricted, but it is preferable to select the animal by considering the adaptability with the parent cells used in cell fusion. Generally, an animal belonging to Rodentia, Lagomorpha, or Primates is used.

45 [0106] As animals belonging to Rodentia, for example, mice, rats, hamsters, and such are used. As animals belonging to Lagomorpha, for example rabbits, as Primates, for example monkeys, are used. As monkeys, monkeys of the infraorder Catarrhini (Old World Monkeys), for example, cynomolgus monkeys, rhesus monkeys, sacred baboons, chimpanzees, etc., are used.

50 [0107] To immunize animals with the sensitizing antigen, well-known methods may be used. For example, the sensitizing antigen is generally injected into mammals intraperitoneally or subcutaneously. Specifically, the sensitizing antigen is suitably diluted, suspended in physiological saline or phosphate-buffered saline (PBS), mixed with a suitable amount of a general adjuvant if desired, for example, with Freund's complete adjuvant, emulsified and injected into the mammal. Thereafter, the sensitizing antigen suitably mixed with Freund's incomplete adjuvant is preferably given several times every four to 21 days. A suitable carrier can also be used when immunizing an animal with the sensitizing antigen. After the immunization, the elevation in the serum antibody level is detected by usual methods.

55 [0108] Polyclonal antibodies against the protein of the invention can be obtained as follows. After verifying that the desired serum antibody level has been reached, blood is withdrawn from the mammal sensitized with the antigen. Serum is isolated from this blood using well-known methods. The serum containing the polyclonal antibody may be used as the polyclonal antibody, or according to needs, the polyclonal antibody-containing fraction may be further isolated from the serum.

60 [0109] To obtain monoclonal antibodies, after verifying that the desired serum antibody level has been reached in the mammal sensitized with the above-described antigen, immunocytes are taken from the mammal and used for cell fusion. At this instance, immunocytes that are preferably used for cell fusion are splenocytes. As parent cells fused with the above immunocytes, preferable are mammalian myeloma cells, more preferable are, myeloma cells that have

attained the feature of distinguishing fusion cells by agents.

[0110] For the cell fusion between the above immunocytes and myeloma cells, for example, the method of Milstein et al. (Galfre, G. and Milstein, C., Methods Enzymol., 1981, 73, 3-46) is basically well known.

[0111] The hybridoma obtained from cell fusion is selected by culturing in a usual selective culture medium, for example, HAT culture medium (hypoxanthine, aminopterin, thymidine-containing culture medium). The culture in this HAT medium is continued for a period sufficient enough for cells (non-fusion cells) other than the objective hybridoma to perish, usually from a few days to a few weeks. Next, the usual limiting dilution method is carried out, and the hybridoma producing the objective antibody is screened and cloned.

[0112] Other than the above method of obtaining a hybridoma by immunizing an animal other than humans with the antigen, a hybridoma producing the objective human antibodies comprising the activity to bind to proteins can be obtained by the method of sensitizing human lymphocytes, for example, human lymphocytes infected with the EB virus, with proteins, protein-expressing cells, or lysates thereof *in vitro*, fusing the sensitized lymphocytes with myeloma cells derived from human, for example U266, having the capacity of permanent cell division (Unexamined Published Japanese Patent Application (JP-A) No. Sho 63-17688).

[0113] Moreover, human antibody against the protein can be obtained using a hybridoma made by fusing myeloma cells with antibody-producing cells obtained by immunizing a transgenic animal comprising a repertoire of human antibody genes with an antigen such as a protein, protein-expressing cells, or a cell lysate thereof WO92/03918, WO93/2227, WO94/02602, WO94/25585, WO96/33735, and WO96/34096).

[0114] Other than producing antibodies by using hybridoma, antibody-producing immunocytes such as sensitized lymphocytes that are immortalized by oncogenes may also be used.

[0115] Such monoclonal antibodies can also be obtained as recombinant antibodies produced by using the gene engineering technique (for example, Borrebaeck, C.A.K. and Larrick, J.W., THERAPEUTIC MONOCLONAL ANTIBODIES, Published in the United Kingdom by MACMILLAN PUBLISHERS LTD, 1990). Recombinant antibodies are produced by cloning the encoding DNA from immunocytes such as hybridoma or antibody-producing sensitized lymphocytes, incorporating this into a suitable vector, and introducing this vector into a host to produce the antibody. The present invention encompasses such recombinant antibodies as well.

[0116] The antibody of the present invention may be an antibody fragment or a modified antibody as long as it binds to the protein of the invention. For example, Fab, F(ab')₂, Fv, or single chain Fv in which the H chain Fv and the L chain Fv are suitably linked by a linker (scFv, Huston, J.S. et al., Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 5879-5883) can be given as antibody fragments. Specifically, antibody fragments are produced by treating an antibody with an enzyme, for example, papain, pepsin, etc. or by constructing a gene encoding an antibody fragment, introducing this into an expression vector, and expressing this vector on suitable host cells (for example, Co, M.S. et al., J. Immunol., 1994, 152, 2968-2976; Better, M. and Horwitz, A.H., Methods Enzymol., 1989, 178, 476-496; Pluckthun, A. and Skerra, A., Methods Enzymol., 1989, 178, 497-515; Lamoyi, E., Methods Enzymol., 1986, 121, 652-663; Rousseaux, J. et al., Methods Enzymol., 1986, 121, 663-669; Bird, R.E. and Walker, B.W., Trends Biotechnol., 1991, 9, 132-137).

[0117] As a modified antibody, an antibody bound to various molecules such as polyethylene glycol (PEG) can be used. The present antibody encompasses such modified antibodies as well. To obtain such a modified antibody, chemical modifications are done to the obtained antibody. These methods are already established in the field.

[0118] The antibody of the invention may be obtained as a chimeric antibody comprising non-human antibody-derived variable region and a human antibody-derived constant region, or as a humanized antibody comprising non-human antibody-derived complementarity determining region (CDR), and human antibody-derived framework region (FR) and a constant region.

[0119] Antibodies thus obtained can be purified till uniform. The separation and purification methods for separating and purifying the antibody used in the present invention may be any method usually used for proteins, and is not in the least limited. Antibody concentration of the above mentioned antibody can be assayed by measuring the absorbance, or by the enzyme-linked immunosorbent assay (ELISA), etc.

[0120] Also, as methods that assay the antigen-binding activity of the antibody of the invention, ELISA, enzyme immunoassay (EIA), radio immunoassay (RIA), or fluorescent antibody method can be given. For example, when using ELISA, the protein of the invention is added to a plate coated with the antibody of the invention, and next, the objective antibody sample, for example, culture supernatants of antibody-producing cells, or purified antibodies are added. Then, secondary antibody recognizing the antibody, which is labeled by alkaline phosphatase and such enzymes, is added, the plate is incubated and washed, and absorbance is measured to evaluate the antigen-binding activity after adding an enzyme substrate such as p-nitrophenyl phosphate. As the protein, a protein fragment, for example, a fragment comprising a C terminus, or a fragment comprising an N terminus may be used. To evaluate the activity of the antibody of the invention, BIACore (Pharmacia) may be used.

[0121] By using these methods, the antibody of the invention and a sample presumed to contain the protein of the invention are contacted, and the protein of the invention is detected or assayed by detecting or assaying the immune complex of the above-mentioned antibody and protein.

[0122] A method of detecting or assaying the protein of the invention is useful in various experiments using proteins as it can specifically detect or assay the proteins.

5 [0123] The present invention also encompasses a DNA specifically hybridizing to a DNA comprising a nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, 20, and 22 to 27 or its complementary DNA, and comprising at least 15 nucleotides. Namely, a probe that can selectively hybridize to the DNA encoding the protein of the invention, or a DNA complementary to the above DNA, a nucleotide or nucleotide derivative, for example, antisense oligonucleotide, ribozyme, and such are included.

10 [0124] The present invention also encompasses an antisense oligonucleotide that hybridizes to any portion of any one of the nucleotide sequences shown in, for example, SEQ ID NOs: 2, 4, 6, 8, 20, and 22 to 27. This antisense oligonucleotide is preferably one against at least 15 continuous nucleotides in any one of the nucleotide sequences of SEQ ID NOs: 2, 4, 6, 8, 20, and 22 to 27. More preferable is the above-mentioned antisense oligonucleotide against the above-mentioned at least 15 continuous nucleotides containing a translation start codon.

15 [0125] Derivatives or modified products of antisense oligonucleotides can be used as antisense oligonucleotides. As such modified products, for example, lower alkyl phosphonate modifications such as methyl-phosphonate-type or ethyl-phosphonate-type, phosphorothioate or phosphoroamidate-modified products, etc. may be used.

[0126] The term "antisense oligonucleotide(s)" as used herein means, not only those in which the nucleotides corresponding to those constituting a specified region of a DNA or mRNA are entirely complementary, but also those having a mismatch of one or more nucleotides, as long as the DNA or mRNA and the oligonucleotide can selectively and stably hybridize with the nucleotide sequence of SEQ ID NO: 1.

20 [0127] "Selectively and stably hybridize" means that significant cross hybridization with DNA encoding other proteins does not occur under usual hybridization conditions, preferably under stringent hybridization conditions. Such DNAs are indicated as those having, in the "at least 15 continuous nucleotide" sequence region, a homology of at least 70% or higher, preferably 80% or higher, more preferably 90% or higher, even more preferably 95% or higher nucleotide sequence homology. The algorithm stated herein can be used to determine homology. Such DNA is useful as a probe for detecting or isolating DNA encoding the protein of the invention, or as a primer for amplification as described in Examples below.

25 [0128] The antisense oligonucleotide derivative of the present invention acts upon cells producing the protein of the invention by binding to the DNA or mRNA encoding the protein to inhibit its transcription or translation, and to promote the degradation of mRNA, and has an effect of suppressing the function of the protein of the invention by suppressing the expression of the protein.

30 [0129] The antisense oligonucleotide derivative of the present invention can be made into an external preparation such as a liniment and a poultice by mixing with a suitable base material, which is inactive against the derivatives.

[0130] Also, as needed, the derivatives can be formulated into tablets, powders, granules, capsules, liposome capsules, injections, solutions, nose-drops, and freeze-dried agents by adding excipients, isotonic agents, solubilizers, stabilizers, preservatives, pain-killers, etc. These can be prepared using the usual methods.

35 [0131] The antisense oligonucleotide derivative is given to the patient by directly applying onto the ailing site, by injecting into a blood vessel, etc. so that it will reach the ailing site. An antisense-mounting material can also be used to increase durability and membrane-permeability. Examples are, liposome, poly-L lysine, lipid, cholesterol, lipofectin, or derivatives of these.

40 [0132] The dosage of the antisense oligonucleotide derivative of the present invention can be adjusted suitably according to the patient's condition and used in desired amounts. For example, a dose range of 0.1 to 100 mg/kg, preferably 0.1 to 50 mg/kg can be administered.

45 [0133] The antisense oligonucleotide derivative of the present invention is useful in inhibiting the expression of the protein of the invention, and therefore is useful in suppressing the biological activity of the protein of the invention. Also, expression-inhibitors comprising the antisense oligonucleotide derivative of the present invention are useful because of their capability to suppress the biological activity of the protein of the invention.

Brief Description of the Drawings

50 [0134]

Figure 1 is a schematic diagram showing the results of BlastX search where the query was 180 nucleotides of 40861-41040 including 40952-40966, the only probe sequence within the AC002303. "#": For only NR8 the number was indicated by the nucleotide number. The underline of the NR8 sequence shows the portion corresponding to the exon. Other underlined sequences show identical amino acids.

55 Figure 2 is a schematic diagram showing the results of BlastX scanning of 180 nucleotides in both the 5' and 3' directions, where the search centered on the 180 nucleotides of 40861-41040 containing 40952-40966, the only probe sequence within the AC002303.

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Figure 3 shows the electrophoresis results of the amplification done by the RT-PCR method for the combinations of SN1/AS1, SN1/AS2, SN2/AS1, and SN2/AS2 primers using human fetal liver and skeletal muscle cDNA as templates.

5 Figure 4 shows the electrophoretic results of the 5'-RACE method and 3'-RACE method using human fetal liver cDNA as the template.

Figure 5 shows the nucleotide sequence and the amino acid sequence of NR8 α cDNA. The arrows show the positions of primers used for RT-PCR. They are, SN1 (798-827), SN2 (894-923), AS2 (1055-1026), and AS1 (1127-1098) from the 5' side, in their order. For two bases at the 5' end of AS1, AC, which is derived from the genomic sequence, was used in place of CT.

10 Figure 6 is the continuation of Fig. 5 showing the nucleotide sequence and the amino acid sequence of NR8 α cDNA.

Figure 7 shows the nucleotide sequence and the amino acid sequence of NR8 β cDNA. Two possible open reading frames (ORF) are shown.

15 Figure 8 is the continuation of Fig. 7 showing the nucleotide sequence and the amino acid sequence of NR8 β cDNA.

Figure 9 shows the nucleotide sequence and the amino acid sequence of NR8 γ cDNA. The 177 amino acids inserted by selective splicing are underlined.

Figure 10 is the continuation of Fig. 9 showing the nucleotide sequence and the amino acid sequence of NR8 γ cDNA. The 177 amino acids inserted by selective splicing are underlined.

20 Figure 11 is the continuation of Fig. 10 showing the nucleotide sequence and the amino acid sequence of NR8 γ cDNA.

Figure 12 shows the results of Northern blot analysis of NR8 expression in each organ.

25 Figure 13 is a schematic diagram showing the structure of the NR8 gene. Other repetitives include, (CA) n , (CAGA) n , (TGGA) n , (CATG) n , (GAAA) n , MSTA, AT-rich, MLT1A1, LINE2, FLAM_C, MER63A, and MSTB.

Figure 14 is a schematic diagram showing the structure of expressible proteins constructed in the expression vector.

Figure 15 shows the results of cross PCR, in which the human NR8 primer set was used against a mouse cDNA library. As the size marker, 100 bp DNA Ladder (NEB#323-1L) was used.

30 Figure 16 shows a comparison between amino acid sequences of human and mouse NR8 β . The amino acid sequences where the two coincide are shadowed. Also, cysteine residues conserved in other hemopoietin receptors are displayed in boldface type within the sequence.

Figure 17 shows a comparison between amino acid sequences of human and mouse NR8 γ . The amino acid sequences where the two coincide are shadowed. Also, cysteine residues conserved in other hemopoietin receptors and the WSXWS-Box are displayed in boldface type within the sequence.

35 Figure 18 shows the results of NR8 gene expression analysis in each mouse organ using the RT-PCR method. The size marker, 100 bp DNA Ladder (NEB#323-1L), is shown on the either sides of the lane. A 320 bp target gene has been detected in all organs.

40 Figure 19 shows the results of NR8 gene expression analysis in each mouse organ using the Northern blotting method (left). An approximately 4.2 kb transcript was intensely detected in the testis only. Mouse β -actin was detected in the same blot as a positive control (right).

Best Mode for Carrying Out the Invention

45 [0135] The present invention shall be described in detail below with reference to examples, but is not be construed as being limited thereto.

Example 1: Two step Blast Search

50 [0136] Probe sequences (256 types) comprising the tggag(t/c)nnnntggag(v/c) (where n is an arbitrary nucleotide) as the oligonucleotide encoding the Trp-Ser-Xaa-Trp-Ser motif were designed. These sequences enable the detection of almost all known hemopoietin receptors, except for the EPO receptor, TPO receptor, and the mouse IL6 receptor. Using each sequence as the query, the GenBank nr database was searched using the BlastN (Advanced BlastN 2.0.4) program. Default values (Descriptions=100, Alignments=100) were used as parameters for the search, except for making the expectation value 100.

55 [0137] Since approximately 500 clones that completely matched the probe sequences were obtained as a result of the primary search, among these, a 180-residue nucleotide sequence of human genome-derived clones (cosmid, BAC, and PAC) containing the probe sequence in approximately the center was excised. Next, using this 180-residue nucle-

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otide sequence as the query, the nr database was searched again using the BlastX (Advanced BlastX 2.0.4) program to search the homology of the amino acid sequence around the probe sequence with known hemopoietin receptors.

[0138] Default values were used as parameters for the search, except for making the expectation value 100. However, when extremely large number of hits were obtained (caused by the Alu sub family that is a high repetitive sequence), it was often difficult to observe hits for known hemopoietic receptors. Therefore, to maximize the sensitivity in such cases, a value of "Expect=1000, Descriptions=500, Alignments=500" was used.

[0139] As a result of the secondary search by BlastX, 28 clones hit one or more known hemopoietin receptors (Table 1 to Table 8).

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Table 1

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Probe	Xaa	Accession No.	Hit site	Locus	blastx (expect=100)
TGGAGTAAATTGGAGC	Aen	U95016	30692 tggatataatggcgt 30678	1p34.1-1p36	mIL1R(Composite), U031
TGGAGCTGATGGAGC	***	Z97987	140006 tggatgtatggatgt 139992	1p36.2-36.3	Unel, Lau Zip p40,
TGGAGCAAGCTGGAGC	Ser	AK028268	39931 tggatgtatggatgt 39917	1q21	metoxin
TGGAGCTGCTGGAGC	Cys	AL009051	78023 tggatgtatggcgt 78037	1q23-24	HP-10, semaphorin F, G
TGGAGCACCTGGAGT	Thr	297200	112905 tggatgtatggatgt 112891	1q24	APP enhancer BP, RAR
TGGAGTGCCTGGAGC	Ala	U956226	101031 tggatgtatggatgt 101017	3	CFTIC, TrR
TGGAGTAGATGGAGT	Arg	284495	2567 tggatgtatggatgt 25588	3p21.3	trithorax
TGGAGCTGATGGAGT	***	274023	6225 tggatgtatggatgt 62241	3p21.3	E2ABP, fibronectin, nidogen
TGGAGTTCTGGAGT	Phe	2862275	7291 tggatgtatggatgt 7277	4p16.3	mena, NMDAR
TGGAGCTCCCTGGAGT	Ala	25072	21277 tggatgtatggatgt 21291	4p16.3	crk, AchR, HER3
TGGAGCTGGCTGGAGC	Cys	249897	30266 tggatgtatggcgt 30252	4p16.3	KIT, FLT3, PDGFRA
TGGAGTGTACTGGAGT	Tyr	AC000951	27290 tggatgtatggatgt 27104	5	collagen
TGGAGCTGTGGAGT	Leu	AC004502	48534 tggatgtatggatgt 485220	5	ADAMTS-1, proerdin, etc
TGGAGTAGATGGAGC	***	L81613	2418 tggatgtatggatgt 2404	5	APC, bat2, p53
TGGAGCTGATGGAGT	Val	AC002122	43879 tggatgtatggatgt 43865	5p15.2	Met tRNA synthase
TGGAGTCCATGGAGT	Pro	AC002380	34646 tggatgtatggatgt 34632	5p15.2	N-WASP, enigma
TGGAGCAACTGGAGC	Asn	AC002479	80443 tggatgtatggatgt 80467	5p15.2	NEU, glycoprotein C
TGGAGCTGCTGGAGT	Cys	AC004592	125445 tggatgtatggatgt 125431	5q31	CD22-B
TGGAGTAQCTGGAGT	Ser	AC002993	3721 tggatgtatggatgt 3735	6	glycoprotein
TGGAGTTGCTGGAGT	Cys	AC00226	114578 tggatgtatggatgt 114564	6	G3P REGULON
TGGAGTAGATGGAGT	Ala	Z84490	20244 tggatgtatggatgt 20230	6	Alu, adrenergic receptor

Table 2

Probe	Xaa	Accession No.	Hil site	Locus	blastx (expect<100)
TTGGACCTTCTGAGC	Phe	AC002112	68699 tggatctttggatc 68695	6	IgHv, MYD116
TTGGACGGCTGGAGC	Gly	UB9938	58829 ttagatctttggatc 58816	6p21	myosin HC, csp250.
TGGACACCTCTGGAGC	Val	UB3688	3588 tggatctttggatc 3572	6p21.3	ring finger, BRCA1
TGGAGGTCATGGAGT	Ala	Z98744	38368 tggatctttggatc 38344	6p21.3-22.3	Alu, AD7c-NTP
TGGACATTCTGGAGT	Cys	AL009031	104326 tggatctttggatc 104311	6p22.3-24.1	ACC synthase
TGGAGGTTCTGGAGT	Val	AL008729	21925 tggatctttggatc 21839	6p24	E1A, DUB-2
TGGAGGTTCTGGAGT	Cys	Z98755	69825 tggatctttggatc 69811	6q16.1-21	dynein
TGGAQCTTCTGAQGC	Pho	Z98172	35654 tggatctttggatc 35640	6q21	HGXPTT
TGAGCAGGGTGGAGC	Arg	Z97989	79118 tggatctttggatc 79102	6q21.22	syn syn, alk, yes, arc
TGAGCTTAATGGAGT	***	Z95326	16602 tggatctttggatc 16576	6q22.1-6q22.33	tyrosinase
TGGAGCTCTGGAGC	Ser	Z98049	25650 tggatctttggatc 256786	6q26-q27	collagen, AT3, C1Qb
TGGAGCTCTGGAGT	Ser	AC003090	22268 tggatctttggatc 22082	7p15	ICE
TGGAGCTATGGAGC	Ille	AC004744	22740 tggatctttggatc 22554	7p15-p21	TSH-R, RNABP
TGGAGTAGCTGGAGC	Ser	AC004485	66396 tggatctttggatc 66370	7p15-p21	Hox 2.4, mILL1Ra[ston-1]
TGGAGTCTTGGAGT	Leu	AC004141	3130 tggatctttggatc 3144	7p21-p22	polyprotein
TGGAGCTCTGGAGC	Arg	AC004548	62879 tggatctttggatc 62862	7q11.23-q21.1	NCAM
TGGAGCAACTGGAGT	Asn	AC000064	9170 tggatctttggatc 9184	7q21	glycoprotein A
TGGAGTTACTGGAGT	Tyr	AC003085	87341 tggatctttggatc 87365	7q21-22	GA3RD
TGGAGTTACTGGAGT	Cys	AC000119	65235 tggatctttggatc 65221	7q21-7q22	Nmyc, FGFR
TGGAGTTACTGGAGT	Cys	AC002458	44435 tggatctttggatc 44421	7q21-7q22	FVII, TopoII
TGGAGTACATGGAGC	Thr	AC000059	9977 tggatctttggatc 9963	7q21-7q22	telomerase, NEAT

Table 3

Table 4

Table 5

Probe	Xaa	Accession No.	Hil site	Locus	blastx (expect=100)
TGAGGCCCTGGAGC	Arg	AC004232	34680 tggggccgtggc 34664	16p 13.3	IgLk, AGPR
TTGGAGTACTGGAGC	Thr	AJ003147	161180 tggggactggc 161168	16p 13.3	RanBP2
TTGGAGCTGGAGC	Val	X71874	11320 tggggatggc 11334	16q22.1	collagen a6IV
TOGAGCAATGGAGT	Lys	AC003663	114346 tggggaaatggc 114360	17	beta-D-glucosidase
TOGAGTCTGGAGC	Leu	AC003967	52898 tggggatggc 52884	17	TTE-1, SEX, Rhe,
TGGAGCAATGGAGC	Arg	AC003971	76277 tggggatggc 76263	18	LINK-1, Ter
TGGAGTCATGGAGT	Ala	AD000812	80891 tggggatggc 30806	19	Alu
TGGAGCTGGAGT	Cys	AC004660	10008 tggggatggc 10022	19	Rep1
TGGAGCCCTGGAGT	Pro	AC004490	14389 tggggatggc 14403	19	mucin, atoxin-2, N-WASP
TTGAGTCATGGAGC	Cys	AC003112	18315 tggggatggc 18311	19p 12(N/H)	TIVOR, PHM, OBR-61c
TGGAGCAATGGAGC	Arg	AC004004	89010 tggggatggc 89096	19p 12	PRLE, IL12R, GM-
TTGAGTCATGGAGC	Arg	presumably a pseudogene.....		CSFRb, IL11R(4ntion codon)
TGGAGGCCCTGGAGT	Thr	AD000685	39177 tggggatggc 39183	19p 13.1	IL3Ra(weak, 22 nonWS)
TTGAGCTGGAGT	***	AC002116	21016 tggggatggc 21001	19q13.1	GM-CSERb(many WS atoms)
TTGAGGCCACTGGAGC	Gln	M63796	37184 tggggatggc 37178	19q13.3	Mpc2, Pro rich protein
TTGAGTACTGGAGT	Tyr	AC004605	7622 tggggatggc 7638	19q13.3	NFCP, titin, Jagged 2
TTGAGTCATGGAGC	***	Z93016	31711 tggggatggc 31725	20	Gap junction
TTGAGTCATGGAGT	Gln	LIGHT	31093 tggggatggc 31079	20q12-18.2	semaphorin F, GHS-R, JAK2
TTGAGTCATGGAGT	Ala	AF039907	578 tggggatggc 565	21(NX1)	GL1, IL11R, IL1R(many WS)
TTGAGTCATGGAGT	Val	AG000937	105 tggggatggc 91	21	IgY, Cyt.Oxidase
TTGAGTCATGGAGT				21q	peroxidasin

Table 6

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Probe	Xaa	Accession No.	Hit site	Locus	Blastx (expect=100)
TGGAGTAAATGGAGT	Lys	AP000034	26803 tggatataatgtt 28789	21q11.1	Na/Ca exchanger
TGGAGTAGTGGAGT	Arg	AP000039	24900 tggatgttgtgtt 24014	21q11.1	RNA polymerase
TGGAGTAGTGGAGT	Glu	AP000035	21721 tggatgttgtgtt 21707	21q11.1	smaphorin F
TGGAGTGTGGAGT	Val	AC000038	26194 tggatgttgtgtt 28150	21q11.1	Glycoprotein
TGGAGTGTGGAGT	Ala	AP000046	7204 tggatgttgtgtt 7218	21q11.1	IgV
TGGAGCATTGGAGC	Ile	AP000052	93728 tggatgttgtgtt 93740	21q11.1	Ig H, TCF-3, CETP
TGGAGCCTGGAGC	Leu	AP000037	17681 tggatgttgtgtt 17667	21q11.1	Alu, BCGF
TGGAGTGGGGAGT	Gly	AP000015	48480 tggatgttgtgtt 48494	21q22.2	TPO
TGGAGTGTGGAGT	Thr	Z97055	151632 tggatgttgtgtt 151618	22	semaphorin H, CD44
TGGAGTGTGGAGT	Gly	Z95113	69326 tggatgttgtgtt 69311	22q11.2-pter	ERF
TGGAGTGTGGAGT	Ala	Z93784	36348 tggatgttgtgtt 36362	22q11.2-pter	Alu, NF2
TGGAGQCTGGAGT	Leu	AC002308	130741 tggatgttgtgtt 130727	22q11.2	collagen a1, Na channel
TGGAGTCCTGGAGC	Pro	AC000086	40705 tggatgttgtgtt 40691	22q11.2	ADH, collagen
TGGAGCATGGAGC	Ile	L77669	21088 tggatgttgtgtt 21074	22q11.2	DiGeorge/sleathrin heavy chain 2
TGGAGCACTGGAGC	Ser	AC000092	9817 tggatgttgtgtgtt 98003	22q11.2	IgHV, PC binding
TGGAGCACTGGAGC	Asn	Z96116	64481 tggatgttgtgtgtt 64495	22q12.1	p150, JLARQWSNWSE ²
TGGAGCTAGTGGAGC	***	AC003071	114780 tggatgttgtgtgtt 114784	22q12.1-pter	PQFRb
TGGAGCCCTGGAGC	Pro	Z40802	2675 tggatgttgtgtgtt 2661	22q12-pter	collagen al
TGGAGCTGGAGT	Ser	Z79989	40825 tggatgttgtgtgtt 40839	22q12-pter	collagen al

Table 7

Tabl 8

Probe	Xaa	Accession No.	Hit site	Locus	blasts (expect=100)
TTGGAACTCACTGGAGT	His	AL021706	11982 tggatccatgttggat 11988	Xq21.1-2.33	dopamine receptor
TTGGAGCTGGTGGAGT	Trp	AC000113	119188 tggatccatgttggat 119202	Xq23	DNA repair protein, MHC
TTGGAGCAACTGGAGT	Lys	AF0017262	98212 tggatccatgttggat 98226	Xq28	RNA polymerase
TTGGAGCTGGTGGAGT	Cys	U82671	36792 tggatccatgttggat 36806	Xq28	XTCF-3c
TTGGAGCTCACTGGAGC	Gln	AF011889	144465 tggatccatgttggat 144451	Xq28	GHRHR, Werner Synd.
TTGGAGCTTAATGGAGC	***	AF030876	107409 tggatccatgttggat 107395	Xq28	GP41, JLR3
TTGGAGCACTGGAGC	Phe	AC002531	106696 tggatccatgttggat 106712	Alu, hprt	
TTGGAGCACTGGAGC	Ser	AC004474	124745 tggatccatgttggat 124731	EGFR, Smad6	
TTGGAGCTTCTGGAGT				PLCB2	PLCB2 (composite)
TGGAGCTTCTGGAGC	Leu	U26425	12899 tggatccatgttggat 12913	mouse DNA	enviope mRNA (composite)
TGGAGCAACTGGAGT	Asn	U96726	61672 tggatccatgttggat 61658	MHC class II	CFTC, [IL2R ⁺]
TGGAGCTCOCTGGAGC	Pro	[U96726]	22244 tggatccatgttggat 22230	RG20003	I-309, TR, IL2R (nonWS)
TGGAGCAGATGGAGC	Arg	AC002482	14276 tggatccatgttggat 14290	EDH17B2	Large tegument protein
TGGAGCTCTTGGAGC	Ser	U34979	24914 tggatccatgttggat 24928		common B (composite, nonWS)
TGGAGCCTTGGAGC	Leu	Z15025	6359 tggatccatgttggat 6373	Bat2	bat2, muclu,
GM-CSFRb (composite, stop)					

Recurdant clones are shadowed. White and underlined letters indicate hits and pseudo-hits, respectively.

[0140] Four clones out of these 28 clones (AC002303, AC003112, AL008637, and AC004004) hit several known hemopoietin receptors, however, AC004004 was excluded as it has a stop codon downstream three amino acids of the

Trp-Ser-Xaa-Trp-Ser motif. Among the three remaining clones, AL008637 was thought to be a known receptor, GM-CSF receptor β . AC002303 is the BAC clone CIT987-SKA-670B5 derived from the 16p12 region of human chromosome no. 16 registered by TIGR group on June 19, 1997 and comprises the full-length of 131530 base pairs (Lamerdin, J.E., et al., GenBank Report on AC003112, 1997).

5 [0141] As shown in Fig. 1, a BlastX search (query: 180 nucleotides of 40861-41040 including tggagtgaatggagt (40952-40966), the only probe sequence within the AC002303) revealed that numerous hemopoietin receptors starting with the TPO receptor and leptin receptor show an evident homology, however, there were no known, database-registered hemopoietin receptors that completely matched the query sequence. Also, a BlastX scanning was done under the above conditions, by excising a sequential 180-residue nucleotide sequence in both the 5' and 3' directions, centering 10 on the 180-residue nucleotide sequence mentioned above, and when this was used as a query, two sequences having a homology to known hemopoietin receptors were found in the regions 39181-39360 and 42301-42480, and were thought to be other exons of the same gene (Fig. 2).

15 [0142] A Pro-rich motif PAPPF was conserved in the 39181-39360 site, and a Box 1 motif in the 42301-42480 site. The 3' side exon adjacent to the exon containing the Trp-Ser-Xaa-Trp-Ser motif has a transmembrane domain, and this domain has a low homology with other hemopoietin receptors, and was not detected by the BlastX scan. These results suggested the possibility of a novel hemopoietin receptor gene existing in the above-described BAC clone CIT987-SKA-670B5.

Example 2: Search for NR8 expressing tissues using RT-PCR

20 [0143] Pseudogenes have been reported to exist in several hemopoietin receptors (Kermouni, A. et al., Genomics, 1995, 29 (2) 371-382; Fukunaga, R. and Nagata, S., Eur. J. Biochem., 1994, 220, 881-891). To verify that NR8 is not a pseudogene, and with the objective of identifying NR8 expressing tissues, transcripts of the NR8 gene were searched by RT-PCR method.

25 [0144] In the AC002303 sequence of the above-described BAC clone, several exon regions widely conserved at the amino acid translation level in known cytokine receptors were surmised, and on the sequence of the surmised exon region, the following primers were synthesized. (See Fig. 5 for the location of each primer.)

30 NR8-SN1; 5'- CCG GCT CCC CCT TTC AAC GTG ACT GTG ACC -3' (SEQ ID NO: 9)
 NR8-SN2; 5'- GGC AAG CTT CAG TAT GAG CTG CAG TAC AGG -3' (SEQ ID NO: 10)
 NR8-AS1; 5'- ACC CTC TGA CTG GGT CTG AAA GAT GAC CGG -3' (SEQ ID NO: 11)
 NR8-AS2; 5'- CAT GGG CCC TGC CCG CAC CTG CAG CTC ATA -3' (SEQ ID NO: 12)

35 [0145] Using the Human Fetal Multiple Tissue cDNA Panel (Clontech #K1425-1) as the template, RT-PCR was attempted using combinations of the above primers. Advantage cDNA Polymerase Mix (Clontech #8417-1) was used for the PCR, which was conducted under the conditions below using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler.

40 [0146] Namely, the PCR conditions were, 94°C for 4 min, 5 cycles of "94°C for 20 sec, 72°C for 3 min," 5 cycles of "94°C for 20 sec, 70°C for 3 min," 28 cycles of "94°C for 20 sec, 68°C for 3 min," 72°C for 4 min, and completed at 4°C.

45 [0147] From the primer locations shown in Fig. 5, amplifications of bands sized 330 bp, 258 bp, 234 bp, and 162 bp can be expected from the combinations of SN1/AS1, SN1/AS2, SN2/AS1, and SN2/AS2. When evaluated using human fetal liver, brain, and skeletal muscle cDNA as the template, clear bands having the anticipated sizes were obtained in the fetal liver only with the respective primer combinations (Fig. 3).

50 [0148] An amplification was not seen at all for fetal brain cDNA, and a band of about 650 bp and a broad band of 400 to 500 bp were observed for fetal skeletal muscle cDNA. However, since the band sizes for skeletal muscle cDNA remained constant even when different combinations of primers were used, it is thought that these bands were non-specific amplifications due to some reason.

55 [0149] The obtained PCR product was subcloned to pGEM-T Easy vector (Promega #A1360), and the nucleotide sequence was determined. The recombination of PCR products to the pGEM-T Easy vector was done by T4 DNA Ligase (Promega #A1360) reacted at 4°C for 12 hr. The genetic recombinant between the PCR product and pGEM-T Easy vector was obtained by transforming *E.coli* strain DH5 α (Toyobo #DNA-903).

[0150] For the selection of the genetic recombinant, Insert Check Ready (Toyobo #PIK-101) was used. The dRhodamine Terminator Cycle Sequencing Kit (ABI/Perkin Elmer #4303141) was used for determining the nucleotide sequence, and analysis was done using the ABI PRISM 377 DNA Sequencer. As a result of determining the nucleotide sequences of all inserts of the 10 independent clones of genetic recombinants, all clones were found to comprise a single nucleotide sequence. These obtained sequences were verified to be partial nucleotide sequences of NR8.

Example 3: Full-length cDNA cloning by the 5' and 3'-RACE methods

[0151] Using the thus-obtained fetal liver-derived cDNA, 5' and 3'-RACE methods were conducted to obtain full-length cDNA (Fig. 4).

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3-1) 5'-RACE method

[0152] 5'-RACE PCR was performed using the above-mentioned NR8-AS1 primer for primary PCR, and NR8-AS2 primer for secondary PCR. Human Fetal Liver Marathon-Ready cDNA Library (Clontech #7403-1) was used as the template and Advantage cDNA Polymerase Mix for the PCR experiment. As a result of PCR under the following conditions using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, two types of PCR products were obtained, which have different sizes through selective splicing.

[0153] Primary PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 72°C for 4 min," 5 cycles of "94°C for 20 sec, 70°C for 4 min," 28 cycles of "94°C for 20 sec, 68°C for 4 min," 72°C for 4 min, and completed at 4°C.

[0154] Secondary PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 70°C for 3 min 30 sec," 28 cycles of "94°C for 20 sec, 68°C for 3 min 30 sec," 72°C for 4 min, and completed at 4°C.

[0155] Both types of PCR products obtained were subcloned to pGEM-T Easy vector as mentioned earlier, and the nucleotide sequences of all inserts were determined for the 16 independent clones of genetic transformants. As before, the dRhodamine Terminator Cycle Sequencing Kit was used for determining the nucleotide sequence, and analysis was done using the ABI PRISM 377 DNA Sequencer. As a result, the clones can be divided into two groups, one having 14 clones, and the other having 2 clones, by the length of the base pairs and the differences in sequence (though described later, the differences lie in the products due to selective splicing, and the group of 14 independent clones comprises the sequence corresponding to exon 5 in the genomic sequence, and the remaining group of two independent clones does not have this sequence).

25

3-2) 3'-RACE method

[0156] 3'-RACE PCR was performed using the above-mentioned NR8-SN1 primer for primary PCR, and NR8-SN2 primer for secondary PCR. Human Fetal Liver Marathon-Ready cDNA Library was used as the template similar to 5'-RACE PCR, and Advantage cDNA Polymerase Mix for the PCR experiment. As a result of conducting PCR under the conditions shown in 3-1), a single band PCR product was obtained.

[0157] The obtained PCR product was subcloned to pGEM-T Easy vector as above, and the nucleotide sequences of all inserts of the 12 independent clones of genetic recombinants were determined. As before, the dRhodamine Terminator Cycle Sequencing Kit was used for determining the nucleotide sequence, and the sequences determined were analyzed using the ABI PRISM 377 DNA Sequencer. As a result, all 12 independent clones showed a single nucleotide sequence.

[0158] As a result of analyzing the nucleotide sequence of the fragments (approximately 1.1 kb and 1.2 kb) amplified by 5'-RACE and 3'-RACE, respectively, it was conceived that the approximately 260 bp of each fragment overlap and extend to the 5' side and 3' side, and contain almost the full-length of NR8 mRNA. These were joined to make a full-length cDNA (NR8 α) (Fig. 5 and Fig. 6). The plasmid containing the NR8 α cDNA (SEQ ID NO: 2) was named pGEM-NR8 α , and *E.coli* containing the plasmid has been internationally deposited at the National Institute of Bio-Science and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the accession number FERM BP-6543 since October 9, 1998 according to the Budapest Treaty.

[0159] As shown in Fig. 5 and Fig. 6, in the ORF of NR8 α cDNA, the Met starting from nucleotide no. 441 is thought to be the start codon due to the presence of an inframe stop codon 39 bp upstream, and completes with two stop codons starting from nucleotide no. 1524. It has the features of, from the N terminus in order, a typical secretion signal sequence, a domain thought to be the ligand binding site containing a Cys residue conserved in other hemopoietic receptor members, a Pro-rich motif, Trp-Ser-Xaa-Trp-Ser motif, a transmembrane domain, a Box 1 motif thought to be involved in signal transduction, and such features of hemopoietin receptors. From the above results, the NR8 gene was thought to encode a novel hemopoietin receptor.

[0160] Analysis of fragments amplified by the RACE method suggested the presence of a splice variant. As a result of nucleotide sequence analysis, this variant was revealed to be lacking approximately 150 bp including the above-described Pro-rich motif of NR8 α . Moreover, as a result of comparing AC002303 sequence with NR8 α , and carrying out analogy of exons/introns (Table 9), the above-described variant was thought to be deficient of the 5th exon due to selective splicing.

Table 9

Exon	# in AC002303	# in NR8	Characteristics
1	<1	: 1-424	inframe stop codon
2	26334-26398	: 425-489	start codon, signal peptide
3	30625-30727	: 490-592	conserved Cys residue
4	33766-33965	: 593-792	conserved Cys residue, N-glycosylation site
5	39240-39394	: 793-947	Pro-rich motif (PAPPF), N-glycosylation site
6	40820-40997	: 948-1125	GWSEWSdp motif
7	41455-41554	: 1126-1225	transmembrane domain
8	42285-42366	: 1226-1307	Box1 (IWAVPSP)
9a	44812-44909	: 1308-1405*	connects to exon 10, Box2-like sequence (PSTLEVYSCH), nontypical exon/intron boundary
9b	44812-45922<	: 1308-2465**	double stop codons, Box2-like sequence (PSTLEVYSCH, PABELVESDG), polyA
10	45441-45922<	: 1406-1934*	double stop codons, polyA

NR8 α^* : exons 1+2+3+4+5+6+7+8+9a+10NR8 β : exons 1+2+3+4+6+7+8+9a+10

(two alternative reading frames for soluble-type and transmembrane(-signal)-type)

NR8 γ^* : exons 1+2+3+4+5+6+7+8+9b

[0161] This variant (NR8 β) can encode a soluble receptor in the truncated form by the joining of the 6th exon directly to the 4th exon and causing a frame shift. The boundary between the exons and the introns takes a consensus

sequence in most cases, but the boundary between the 9th exon (Exon 9a) and the 9th intron is the only boundary that takes a different sequence from the consensus sequence (nag/gtgagt, etc.), being acc/acggag. The plasmid comprising NR8 β cDNA (SEQ ID NO: 4) was named pGEM-NR8 β , and *E.coli* comprising the plasmid has been internationally deposited at the National Institute of BioScience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the accession number FERM BP-6544 since October 9, 1998 according to the Budapest Treaty.

Example 4: Northern blotting

[0162] In order to analyze the distribution and mode of NR8 gene expression in each human organ and human cancer cell lines, Northern blot analysis was done using the cDNA encoding the full-length NR8 α protein prepared based on all the cDNA fragments obtained in Example 3 as a probe. The probe was prepared using Mega Prime Kit (Amersham, cat#RPN1607) by radiolabeling it with [α -³²P] dCTP (Amersham, cat#AA0005).

[0163] As Northern blots, Human Multiple Tissue Northern (MTN) Blot (Clontech #7760-1), Human MTN Blot IV (Clontech #7766-1), and Human Cancer Cell Line MTN Blot (Clontech #7757-1) were used. Express Hyb Hybridization Solution (Clontech #8015-2) was used for hybridization.

[0164] Hybridization conditions were: a prehybridization at 68°C for 30 min, followed by hybridization at 68°C for 14 hr. After washing under the following conditions, the blots were exposed to Imaging Plate (FUJI#BAS-III), and the gene expression of NR8 mRNA was detected by the Image Analyzer (FUJIX, BAS-2000 II). Washing conditions were: (1) 1x SSC/0.1% SDS, at room temperature for 5 min; (2) 1x SSC/0.1% SDS, at 50°C 30 min; and (3) 0.1x SSC/0.1% SDS, at 50°C 30 min.

[0165] Fig. 12 shows the results of Northern blot analysis of NR8 expression in each organ. A total of three different-sized mRNA, one 5kb-sized and two 3 to 4kb sized, were detected in human adult lung, spleen, thymus, skeletal muscle, pancreas, small intestines, peripheral leucocytes, and uterus. A similar examination of various cell lines including hemopoietic cell lines showed similar sized bands in two cell lines, the promyeloid leukemic cell line HL60 and Burkett's lymphoma-derived Raji.

Example 5: Plaque screening

[0166] Northern blot analysis of NR8 gene expression detected at least three types of specific mRNA bands with different sizes in each human organ and in each human cancer cell line for which NR8 gene expression was seen. However, the inventors had succeeded in isolating only two types of selective splicing variants, namely NR8 α and NR8 β genes, in the above-described Examples. Therefore, the inventors performed plaque screening with the objective of isolating the gene of the third selective splicing variant. Human Lymph Node (Clontech, cat#HL5000a) that showed a strong NR8 gene expression in the above-mentioned Northern analysis results, was used as the cDNA library. The probe used was NR8 α cDNA fragment, which was radio-labeled by [α -³²P] dCTP (Amersham, cat#AA0005) using the Mega Prime Kit (Amersham, cat#RPN1607). Approximately 7.2 x 10⁵ plaques of Human Lymph Node cDNA Library were blotted onto a Hybond N (+) (Amersham, cat#RPN303B) charged nylon membrane to conduct primary screening. Rapid Hybridization Buffer (Amersham, cat#RPN1636) was used for the hybridization. Hybridization conditions were: a prehybridization at 65°C for 1 hr, followed by hybridization at 65°C for 14 hr. After washing under the conditions, (1) 1x SSC/0.1% SDS, at room temperature for 15 min; (2) 1x SSC/0.1% SDS, at 58°C 30 min; and (3) 0.1x SSC/0.1% SDS, at 58°C 30 min, the membrane was exposed to an X-ray film (Kodak, cat#165-1512) to detect NR8 positive plaques.

[0167] As a result, positive or pseudo-positive 16 independent clones were obtained. A similar secondary screening was done for the 16 clones obtained from the primary screening to successfully isolate plaques of NR8 positive 15 independent clones. The inserts of these 15 clones were amplified by PCR through a pair of primers located in both ends of the λ gt10 vector cloning site. Advantage cDNA polymerase Mix (Clontech #8417-1) was used for the PCR reaction conducted using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, under the following experiment conditions. Namely, 94°C for 4 min, 5 cycles of "94°C for 20 sec, 70°C for 4 min," 30 cycles of "94°C for 20 sec, 68°C for 4 min," 72°C for 4 min, and completed at 4°C.

[0168] Similar to above, the obtained PCR products were subcloned to pGEM-T Easy vector, and the nucleotide sequence of the inserts were determined using the BigDye Terminator Cycle Sequencing SF Ready Reaction Kit (ABI/Perkin Elmer#4303150), and analyzed by the ABI PRISM 377 DNA Sequencer. As a result, among the 15 clones obtained, at least two clones showed an insertion of 177 amino acids flanking the NR8 α C terminus, and since this portion derives from the 9th intron of the NR8 gene and is removed by splicing in NR8 α , this 3rd selective splicing variant was named NR8 γ . The plasmid containing the NR8 γ cDNA (SEQ ID NO: 8) was named pGEM-NR8 γ , and *E.coli* containing the plasmid has been internationally deposited at the National Institute of BioScience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan) under the accession number FERM BP-6545 since October 9, 1998 according to the Budapest Treaty.

[0169] Among the 15 clones obtained here, four clones other than the two mentioned above were further selected, and their nucleotide sequences were analyzed. As a result, among the six clones selected, two clones had the NR8 β nucleotide sequence, and all the remaining four clones had the NR8 γ nucleotide sequence. Therefore, the six clones for which the nucleotide sequence was analyzed did not contain the NR8 α sequence. The NR8 γ cDNA clones for which the nucleotide sequences were determined included those having 3'-UTR (3UTR-2) in which a poly-A tail is added to the site elongated 483 bp from the 3'-UTR of NR8 α obtained by the 3'-RACE method (3UTR-1), and those having 3'-UTR (3UTR-3) in which a poly-A tail is added to the site elongated 2397 bp from the 3'-UTR of NR8 α . On the other hand, the two clones of NR8 β for which the nucleotide sequence was decided above, both contained the nucleotide sequence of 3UTR-3. In Table 10 below, the 3' end non-translation region sequences contained in the cDNA clones thus far obtained are summarized. Also, the nucleotide sequences of 3UTR-1, 3UTR-2, and 3UTR-3 following the translation stop codon of NR8 γ cDNA sequence are shown in SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 25, respectively.

[0170] Moreover, the nucleotide sequences of 3UTR-B1 and 3UTR-B3 following the translation stop codon of NR8 β cDNA sequence are shown in SEQ ID NO: 26 and SEQ ID NO: 27, respectively.

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Table 10

NR8 cDNA clone	3'-UTR sequence
NR8 α	3UTR-1
NR8 β	3UTR-B1, 3UTR-B3
NR8 γ	3UTR-1, 3UTR-2, 3UTR-3

[0171] The nucleotide sequences thus obtained revealed that the gene transcripts of NR8 can encode various different sizes not only due to the differences in selective splicing, but also due to the length of the 3' end non-translation region sequence. This may adequately explain the presence of various-sized transcripts detected by Northern blot analysis.

30 Example 6: Ligand screening

6-1) Construction of NR8 chimeric receptor

[0172] A screening system was constructed for searching a ligand that can specifically bind to NR8, namely, a novel hemopoietin. First, the cDNA sequence encoding the extracellular region of NR8 α (the amino acid sequence of SEQ ID NO: 1; from the 1st Met to the 228th Glu) was amplified by PCR, and this DNA fragment was bound to DNA fragments encoding the transmembrane region and the intracellular region of a known hemopoietin receptor to prepare a fusion sequence encoding a chimeric receptor. As described above, there were several candidates for the partner, the known hemopoietin receptor, and among them, the human TPO receptor (Human MPL-P) was selected. Namely, after amplifying the DNA sequence encoding the intracellular region that includes the transmembrane region of the human TPO receptor by PCR, this sequence was bound to the cDNA sequence encoding the extracellular region of NR8 α in frame, and inserted into a plasmid vector expressible in mammalian cells. The expression vector constructed was named pEF-NR8/TPO-R. A schematic diagram of the structure of the constructed NR8/TPO-R chimeric receptor is shown in Fig. 14, and the nucleotide sequence of the chimeric receptor and the expressible amino acid sequence encoded by it are shown in SEQ ID NOs: 13 and 14, respectively. Together with an expression vector pSV2bsr (Kaken Pharmaceutical Co., Ltd.) containing Blastcidin S resistant gene, the NR8/TPO-R chimeric receptor-expressing vector was introduced into the growth factor-dependent cell line Ba/F3, and forcedly expressed. Gene-introduced cells were selected by culturing with 8 μ g/ml of Blastcidin S hydrochloride (Kaken Pharmaceutical Co., Ltd.) and IL-3. By transferring the obtained chimeric receptor-introduced cells to an IL-3-free medium, adding a material expected to contain a target ligand, and culturing, it is possible to conduct a screening that uses the fact that survival/proliferation will be possible only when a ligand that specifically binds to NR8 is present.

6-2) Preparation of NR8/IgG1-Fc soluble fusion protein

[0173] NR8/IgG1-Fc soluble fusion protein was prepared to be used for searching cell membrane-bound ligands, or the detection of soluble ligands through BIACore (Pharmacia) and West-western blotting. A fusion sequence encoding the soluble fusion protein was prepared by binding a DNA fragment encoding the extracellular region of NR8 α (amino acid sequence; from the 1st Met to the 228th Glu) prepared in 5-1 with the DNA fragment encoding the Fc

region of human immunoglobulin IgG1 in frame. A schematic diagram of the structure of the soluble fusion protein encoding the NR8/IgG1-Fc is shown in Fig. 14, and the nucleotide sequence and the expressible amino acid sequence encoded by it in SEQ ID NOs: 15 and 16, respectively. This fusion gene fragment was inserted into a plasmid vector expressible in mammalian cells, and the constructed expression vector was named pEF-NR8/IgG1-Fc. If this pEF-NR8/IgG1-Fc is forcedly expressed in mammalian cells, and after selecting stable gene-introduced cells, the recombinant protein secreted into the culture supernatant can be purified by immunoprecipitation using anti-human IgG1-Fc antibody, or by affinity columns, etc.

6-3) Construction of an expression system of NR8 β and purification of recombinant NR8 β protein

[0174] The recombinant NR8 β protein was prepared to be used for searching cell membrane-bound ligands, or the detection of soluble ligands using BIAcore (Pharmacia) or West-western-blotting. Using the amino acid coding sequence of NR8 β cDNA, the stop codon was replaced by point mutation to a nucleotide sequence encoding an arbitrary amino acid residue, and then, was bound to the nucleotide sequence encoding the FLAG peptide in frame. This bound fragment was inserted into a plasmid vector expressible within mammalian cells, and the constructed expression vector was named pEF-BOS/NR8 β FLAG. Fig. 14 shows a schematic diagram of the structure of the insert NR8 β FLAG within the constructed expression vector. Moreover, the nucleotide sequence of NR8 β FLAG and the expressible amino acid sequence encoded by it are shown in SEQ ID NOs: 17 and 18, respectively. If this pEF-BOS/NR8 β FLAG is forcedly expressed in mammalian cells, and after selecting stable gene-introduced cells, the recombinant protein secreted into the culture supernatant can be immunoprecipitated using anti-FLAG peptide antibody, or may be purified by affinity columns, etc.

Example 7: Isolation of mouse NR8 (mNR8) gene

7-1) The mouse homologous gene using human NR8 primers

[0175] Xenogeneic cross PCR cloning was isolated using the oligonucleotide primers, NR8-SN1 and NR8-SN2 (SEQ ID NOs: 9 and 10) at the sense side (downstream direction) and NR8-AS1 and NR8-AS2 (SEQ ID NOs: 11 and 12) at the antisense side (upstream direction), which were used for isolating full-length cDNA of human NR8. By combining the above-mentioned human NR8 primers, four types of primer sets can be constructed. Namely, using the combinations of "NR8-SN1 vs. NR8-AS1," "NR8-SN1 vs. NR8-AS2," "NR8-SN2 vs. NR8-AS1," and "NR8-SN2 vs. NR8-AS2," and a mouse brain cDNA library (Clontech #7450-1) and a mouse testis cDNA library (Clontech #7455-1) as templates, amplification of cross PCR products was expected. Advantage cDNA Polymerase Mix (Clontech #8417-1) was used for the PCR that was conducted under the conditions below using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler to amplify partial nucleotide sequence that could encode a mouse homologous gene of this receptor.

[0176] Namely, the cross PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 72°C for 1 min," 5 cycles of "94°C for 20 sec, 70°C for 1 min," 28 cycles of "94°C for 20 sec, 68°C for 1 min," 72°C for 4 min, and completed at 4°C.

[0177] As a result, as shown in Fig. 15, an amplification of the cross PCR product was seen when any primer set was used. Also, a much clearer amplification product can be obtained when mouse brain cDNA was used as the template than when mouse testis cDNA was used.

7-2) Determination of the partial nucleotide sequence of the mouse homologous gene corresponding to NR8

[0178] Among the amplification products obtained in 7-1), mouse brain cDNA-derived product was subcloned to pGEM-T Easy vector (Promega #A1360), and the nucleotide sequence was determined. Namely, the PCR product was recombined into pGEM-T Easy vector by using T4 DNA ligase (Promega #A1360) at 4°C for 12 hr, and the resulting product was transfected into *E.coli* strain DH5 α (Toyobo #DNA-903) to obtain the genetic recombinants of the PCR product and pGEM-T Easy vector. For the selection of genetic recombinant, Insert Check Ready Blue (Toyobo #PIK-201) was used. The nucleotide sequence was determined by using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI/Perkin Elmer #4303154), and sequence analysis was done by the ABI PRISM 377 DNA Sequencer. As a result of determining the nucleotide sequence of all inserts of eight independent clones of genetic recombinants, nucleotide sequences derived from the same transcript were obtained, and they were verified to be partial nucleotide sequences of mNR8. The obtained partial nucleotide sequence is shown in SEQ ID NO: 28.

7-3) Design of oligonucleotide primers specific to the mouse NR8 gene

[0179] Based on the partial nucleotide sequence of mNR8 obtained in 7-2), oligonucleotide primers specific to the

mouse NR8 were designed. As shown in the sequence given below, mNR8-SN3 was synthesized in the sense side (downstream direction), and, mNR8-AS3 was synthesized in the antisense side (upstream direction). ABI's 394 DNA/RNA Synthesizer was used for primer synthesis, which was done under 5'-end trityl residue addition conditions. After that, the complete length of the synthesized product was purified by using an OPC column (ABI #400771). These 5 primers contributed towards the 5'-RACE method and the 3'-RACE method described later on.

mNR8-SN3; 5'- TCC AGG CGC TCA GAT TAC GAA GAC CCT GCC -3' (SEQ ID NO: 29)
 mNR8-AS3; 5'- ACT CCA GGT CCC CTG GTA GGA GGA GCC AGG -3' (SEQ ID NO: 30)

10 7-4) Cloning of cDNA corresponding to N terminus by the 5'-RACE method

[0180] To isolate full-length cDNA of mNR8, 5'-RACE PCR was performed using the NR8-AS2 primer (SEQ ID NO: 12) for the primary PCR, and the above-mentioned mNR8-AS3 primer (SEQ ID NO: 30) for secondary PCR. Mouse Brain Marathon-Ready cDNA Library (Clontech #7450-1) was used as the template, and Advantage cDNA Polymerase 15 Mix for PCR experiment. As a result of conducting PCR under the following conditions using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, PCR products of two different sizes were obtained.
 [0181] Primary PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 72°C for 100 sec," 5 cycles of "94°C for 20 sec, 70°C for 100 sec," 28 cycles of "94°C for 20 sec, 68°C for 100 sec," 72°C for 3 min, and completed at 4°C.
 20 [0182] Secondary PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 70°C for 100 sec," 25 cycles of "94°C for 20 sec, 68°C for 100 sec," 72°C for 3 min, and completed at 4°C.
 [0183] Both types of PCR products obtained were subcloned to pGEM-T Easy vector as described above, and the 25 nucleotide sequences were determined. Namely, the PCR products were recombined into the pGEM-T Easy vector with T4 DNA ligase at 4°C for 12 hr, and the resulting product was transfected into *E.coli* strain DH5 α to obtain the genetic recombinant between the PCR product and pGEM-T Easy vector. Also, as mentioned earlier, Insert Check Ready Blue 30 was used for the selection of the genetic recombinant. For the determination of the nucleotide sequence, the BigDye Terminator Cycle Sequencing Ready Reaction Kit was used, and the nucleotide sequence was analyzed by the ABI PRISM 377 DNA Sequencer. The result of determining the nucleotide sequences of all inserts of eight independent clones of genetic recombinants suggests that they could be divided into two groups of four clones each by the base pair length and differences in the sequence. This difference of the products was caused by selective splicing, and both of the obtained sequences were verified to contain the sequence of full-length mNR8 cDNA clone corresponding to the N terminal sequence. The cDNA clone comprising the long ORF containing the exon encoding the Pro-rich region was named mNR8 γ , and the cDNA clone encoding the short ORF that does not have the Pro-rich region was named mNR8 β . These clones correspond to xenogeneic homologous genes of human NR8 γ and human NR8 β , respectively.

35 7-5) Cloning of cDNA corresponding to C terminus using the 3'-RACE method

[0184] To isolate full-length cDNA of mNR8, 3'-RACE PCR was performed using the NR8-SN1 primer (SEQ ID NO: 9) for the primary PCR, and the mNR8-SN3 primer (SEQ ID NO: 29) for secondary PCR. Mouse Brain Marathon-Ready 40 cDNA Library was used as the template, and Advantage cDNA Polymerase Mix for PCR experiment. As a result of conducting PCR under the above-mentioned conditions using the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler, a PCR product of a single size was obtained. The PCR product obtained was subcloned to pGEM-T Easy vector as before according to 7-2), and the nucleotide sequence was determined. As a result of determining the nucleotide sequences of all inserts of four independent clones of genetic recombinants, it was found to contain the sequence of 45 full-length mNR8 cDNA corresponding to the C terminal sequence. By combining the resulting nucleotide sequence determined through this 3'-RACE PCR, and the nucleotide sequence of 5'-RACE PCR products determined in 7-4), the complete nucleotide sequences of the full-length of mNR8 γ and mNR8 β cDNA were finally determined. The determined mNR8 γ cDNA nucleotide sequence and the amino acid sequence encoded by it are shown in SEQ ID NOs: 22 and 21, respectively. The determined mNR8 β cDNA nucleotide sequence and the amino acid sequence encoded by it are 50 shown in SEQ ID NOs: 20 and 19, respectively.
 [0185] When the human and mouse NR8 amino acid sequences were compared, a high homology of 98.9% was seen for NR8 γ , and the homology was 97.2% even for NR8 β . This result strongly suggests the possibility that the same receptor gene has a vital functional responsibility that exceeds species. Fig. 16 shows a comparison between human and mouse NR8 β amino acid sequences. Fig. 17 shows a comparison between human and mouse NR8 γ amino acid 55 sequences.
 [0186] Both the full-length cDNAs of mNR8 γ and mNR8 β finally isolated were able to encode the transmembrane receptor protein comprising 538 amino acids, and the soluble receptor-like protein comprising 144 amino acids, respectively, through a selective splicing similar to human NR8. The structure below shows the characteristics of mNR8 γ . First,

it is presumed that from amino acid no. 1 Met to amino acid no. 19 Gly is a typical secretion signal sequence. Here, since an inframe stop codon exists in the minus 13 position from the 1st Met, this Met residue is presumed to be the translation start codon. Next, from the 25th Cys to the 35th Cys residue is a typical ligand binding site sequence, and the 65th and 109th Cys residues also show the repetitive Cys residue structure conserved in other hemopoietin receptors as well. Next, the Pro-rich region is conserved by the Pro residues repeating at the 120th, 122nd and 123rd positions. From the 214th Trp to 218th Ser residue is a typical WSXWS-Box (WS motif). Following these structural characteristics in the extracellular region, a typical transmembrane domain is seen in the 23 amino acids from the 233rd Gly to the 255th Leu. In the intracellular region that follows, the 271st and 273rd Pro residues are Box-1 consensus sequence (PXP motif) conserved in other hemopoietin receptor members, and these are thought to be deeply involved in signal transduction. Thus, mNR8 γ adequately satisfies the characteristics of hemopoietin receptor members.

5 [0187] On the other hand, for mNR8 β , among the structural characteristics for the above-mentioned extracellular region, the exon sequence encoding the Pro-rich region has been skipped by selective splicing, and directly joins the next exon encoding the WS motif. However, the WSXWS-Box sequence has been excluded from the reading frame by frame shift, and after coding up to 144th Leu, the translation frame completed the next stop codon. Thus, a soluble
10 hemopoietin receptor-like protein that does not have a transmembrane domain is encoded.

15 **Example 8: Expression analysis of mouse NR8 gene**

20 **8-1) Analysis of mouse NR8 gene expression by the RT-PCR method**

25 [0188] To analyze the distribution and mode of NR8 gene expression in each mouse organ, the mRNA was detected by RT-PCR analysis. As primers for this RT-PCR analysis, NR8-SN1 primer (SEQ ID NO: 9) was used as the sense side (downstream direction) primer, and NR8-AS1 primer was used as the antisense side (upstream direction) primer. Mouse Multiple Tissue cDNA Panel (Clontech #K1423-1) was used as the template. Advantage cDNA Polymerase Mix (Clontech #8417-1) and the Perkin Elmer Gene Amp PCR System 2400 Thermalcycler were used for PCR. The target genes were amplified by the PCR reaction under the cycle condition given below.

30 [0189] PCR conditions were 94°C for 4 min, 5 cycles of "94°C for 20 sec, 72°C for 1 min," 5 cycles of "94°C for 20 sec, 70°C for 1 min," 24 cycles of "94°C for 20 sec, 68°C for 1 min," 72°C for 3 min, and completed at 4°C.
35 [0190] The results of RT-PCR are shown in Fig. 18. The NR8 gene was strongly detected in the testis and day 17 embryo, and a constitutive gene expression was seen in all mouse organs and in all mouse tissue-derived mRNA analyzed. By detecting the expression of the house keeping gene G3PDH under the above-mentioned PCR conditions using the mouse G3PDH primer for all the templates used in the analysis, it has been verified beforehand that the number of copies of template mRNA has been normalized (standardized) between samples. The detected RT-PCR product size herein was 320 bp, and this coincides with the size calculated by the determined nucleotide sequence.
40 Therefore, it was thought to be the product of the mouse NR8 specific PCR amplification reaction. To further verify this, the PCR product amplified in the day 17 embryo was subcloned to pGEM-T Easy vector according to 7-2), and the nucleotide sequence was analyzed. The result verified that the PCR product could be a partial nucleotide sequence of mouse NR8, and the possibility that it might be the product of a non-specific PCR amplification was denied.

45 **8-2) Analysis of mouse NR8 gene expression by Northern blotting**

50 [0191] In order to analyze NR8 gene expression in each mouse organ, and with the objective of identifying the NR8 transcription size, gene expression analysis by the Northern blotting method was conducted. Mouse Multiple Tissue Northern Blot (Clontech #7762-1) was used as the blot. Among the 5'-RACE products obtained in 7-4), the mNR8 β cDNA fragment was used as the probe. The probe was radiolabeled with [α -³²P] dCTP (Amersham, cat#AA0005) using Mega Prime Kit (Amersham, cat#RPN1607). Express Hyb Hybridization Solution (Clontech #8015-2) was used for hybridization. After a prehybridization at 68°C for 30 min, the heat-denatured labeled probe was added, and hybridization was conducted at 68°C for 16 hr. After washing under the following conditions, the blot was exposed to Imaging Plate (FUJI #BAS-III), and a mouse NR8 specific signal was detected by the Image Analyzer (FUJIX, BAS-2000 II).

55 [0192] Washing conditions were: (1) 1x SSC/0.1% SDS, at room temperature for 5 min; (2) 1x SSC/0.1% SDS, at 50°C 30 min; and (3) 0.5x SSC/0.1% SDS, at 50°C 30 min.

60 [0193] As a result, as shown in Fig. 19, a strong expression was seen in the mouse testis only, and no gene expression of the same gene was detected in other organs. Here, there is a difference between the results of RT-PCR analysis and Northern blot analysis. Since the detection sensitivity of the Northern method is much lower than RT-PCR, it is thought that mRNA with low expression levels could not be detected. However, results of both analyses coincide in the point that a strong gene expression was detected in the testis. Also, the size of the detected transcript was about 4.2 kb.

65 [0194] Although there was a deviation of the expression levels in each mouse organ analyzed by the Northern method and RT-PCR, the gene expression was widely distributed, being detectable in all the organs analyzed especially

when using RT-PCR. This result contrasts with the human NR8 gene in which the expression was strong only in immunocompetent tissues, hemopoietic tissues, and specific leukemic cell lines, and the significance of this expression is extremely interesting. This means namely the possibilities that in mouse, the NR8 molecule not only is involved in systemic hemopoietic functions, or in immunological responses, and hemopoiesis, but also may be involved in various physiological regulatory mechanisms of the body. Namely, its ligand may be able to function as a hormone-like factor.

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Example 9: Isolation of the NR8 mouse genomic gene by plaque screening

[0195] The present inventors analyzed the genomic structure of mouse NR8 gene and performed plaque hybridization against the mouse genomic DNA library. 129SVJ strain Genomic DNA (Stratagene #946313) constructed in Lambda FIX II was used as the library. This genomic library of approximately 5.0×10^5 plaques was developed and blotted to a Hybond N+(Amersham #RPN303B) charged nylon membrane to perform primary screening. NR8 β cDNA fragment of 5'-RACE products obtained in 7-4) was used as the probe. The probe was radiolabeled with [α - 32 P] dCTP prepared as above-mentioned in 8-2) using the Mega Prime Kit. Express Hyb Hybridization Solution was used for hybridization, and after a prehybridization at 65°C for 30 min, a heat-denatured labeled probe was added, and hybridization was done at 65°C for 16 hr. After washing under the following conditions, the membrane was exposed to an X-ray film (Kodak, cat#165-1512) to detect mouse NR8 positive plaques.

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[0196] Washing conditions were: (1) 1x SSC/0.1% SDS, at room temperature for 5 min; (2) 1x SSC/0.1% SDS, at 58°C 30 min; and (3) 0.5x SSC/0.1% SDS, at 58°C 30 min.

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[0197] As a result, positive, or pseudo-positive 16 independent clones were obtained. When a secondary screening was similarly conducted against these 16 clones obtained by the primary screening, the inventors succeeded in isolating NR8 positive, nine independent plaque clones.

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Industrial Applicability

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[0198] The present invention provides a novel hemopoietin receptor protein "NR8," and the encoding DNA. The present invention also provides, a vector into which the DNA has been inserted, a transformant harboring the DNA, and a method of producing a recombinant protein using the transformant. It also provides a method of screening a compound or a natural ligand that binds to the protein. The NR8 protein of the invention is thought to be related to hemopoiesis, and therefore, is useful in analyzing hemopoietic functions. The protein would also be applied in the diagnosis and treatment of hemopoiesis-associated diseases.

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[0199] Since the expression of mouse NR8 gene was widely distributed in mouse organs, mouse NR8 protein would be involved in various physiological regulatory mechanisms of the body, including the above-mentioned hemopoiesis. Furthermore, by using mouse NR8 protein, it is possible to isolate first the mouse NR8 ligand, and next, the human homologue of the NR8 ligand using the conserved structure of the mouse NR8 ligand. Specifically, after determining the nucleotide sequence of mouse NR8 ligand cDNA, an oligonucleotide primer is designed on this sequence, and using this to conduct cross PCR using the human-derived cDNA library as the template, human NR8 ligand cDNA can be obtained. Alternatively, human NR8 ligand cDNA can be obtained by conducting cross hybridization against human-derived cDNA library using mouse NR8 ligand cDNA as the probe. It is also possible to analyze biological function of the NR8 receptor protein by creating a mouse NR8 gene-deficient mouse using the mouse NR8 gene.

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15	Ser Asp Tyr Glu Asp Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln		
	140	145	150
	155		
20	Tyr Glu Leu Gln Tyr Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro		
	160	165	170
25	Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro		
	175	180	185
30	Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly		
	190	195	200
35	Pro Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp		
	205	210	215
40	Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn		
	220	225	230
	235		
45	Pro His Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala		
	240	245	250
50	Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile		
	255	260	265
55	Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly		
	270	275	280
	Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser		
	285	290	295
60	Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu		
	300	305	310
	315		

Val Tyr Ser Cys His Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu
 320 325 330

5 Thr Glu Leu Gln Glu Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro
 335 340 345

10 Lys Pro Ser Phe Trp Pro Thr Ala Gln Asn Ser Gly Gly Ser Ala Tyr
 350 355 360

15 Ser Glu Glu Arg Asp Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val
 365 370 375

20 Thr Val Leu Asp Ala Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu
 380 385 390 395

25 Asp Asp Gly Tyr Pro Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser
 400 405 410

30 Pro Gly Leu Glu Asp Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser
 415 420 425

35 Cys Gly Cys Val Ser Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly
 430 435 440

40 Ser Leu Leu Asp Arg Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp
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45 Ala Gly Gly Leu Pro Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu
 460 465 470 475

50 Ser Glu Ala Gly Ser Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp
 480 485 490

55 Ser Gly Phe Val Gly Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe
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60 Thr Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp
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cctctgtctg ctgctctgtg ggacacactgc ctggaggccc agctgcccgt catcagagtg 240

25 acaggcttta tgacagccctg attgggtact cgggctgggt gtggatttca accccaggcc 300

tctgcctgtctt ttctcagacc ctcatctgtc acccccacgc tgaaccacgc tgccacccccc 360

30 agaagcccat cagactgccc ccagcacacg gaatggattt ctgagaaaga agccgaaaca 420

35 gaaggccctgt gggagtcagc atg ccg cgt ggc tgg gcc ccc ttg ctc ctg 473
Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu

1 5 10

40 ctg ctg ctc cag gga ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc 521
Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr

15 20 25

45 gat tac ctc cag acg gtc atc tgc atc ctg gaa atg tgg aac ctc cac 569
Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His
30 35 4050 ccc agc acg ctc acc ctt acc tgg caa gac cag tat gaa gag ctg aag 617
Pro Ser Thr Leu Thr Leu Trp Gln Asp Gln Tyr Glu Glu Leu Lys
45 50 55

55

5	gac gag gcc acc tcc tgc agc ctc cac agg tcg gcc cac aat gcc acg Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr 60	65	70	75	665
10	cat gcc acc tac acc tgc cac atg gat gta ttc cac ttc atg gcc gac His Ala Thr Tyr Thr Cys His Met Asp Val Phe His Phe Met Ala Asp 80	85	90		713
15	gac att ttc agt gtc aac atc aca gac cag tct ggc aac tac tcc cag Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln 95	100	105		761
20	gag tgt ggc agc ttt ctc ctg gct gag agc atc aag ccc gct ccc ect Glu Cys Gly Ser Phe Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro 110	115	120		809
25	ttc aac gtg act gtg acc ttc tca gga cag tat aat atc tcc tgg cgc Phe Asn Val Thr Val Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg 125	130	135		857
30	tca gat tac gaa gac cct gcc ttc tac atg ctg aag ggc aag ctt cag Ser Asp Tyr Glu Asp Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln 140	145	150	155	905
35	tat gag ctg cag tac agg aac cgg gga gac ccc tgg gct gtg agt ccg Tyr Glu Leu Gln Tyr Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro 160	165	170		953
40	agg aga aag ctg atc tca gtg gac tca aga agt gtc tcc ctc ctc ccc Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro 175	180	185		1001
45	ctg gag ttc cgc aaa gac tcg agc tat gag ctg cag gtg cgg gca ggg Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly 190	195	200		1049
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10	cct cac ctg ctg ctt ctc ctc ctg ctt gtc ata gtc ttc att cct gcc Pro His Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala 240 245 250	1193
15	ttc tgg agc ctg aag acc cat cca ttg tgg agg cta tgg aag aag ata Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile 255 260 265	1241
20	tgg gcc gtc ccc agc cct gag cgg ttc ttc atg ccc ctg tac aag ggc Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly 270 275 280	1289
25	tgc agc gga gac ttc aag aaa tgg gtg ggt gca ccc ttc act ggc tcc Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser 285 290 295	1337
30	agc ctg gag ctg gga ccc tgg agc cca gag gtg ccc tcc acc ctg gag Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu 300 305 310 315	1385
35	gtg tac agc tgc cac cca cca cgg agc ccc gag tct gac ggt gtg ccc Val Tyr Ser Cys His Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu 320 325 330	1433
40	acg gag cta caa gaa cca gca gag ctg gtg gag tct gac ggt gtg ccc Thr Glu Leu Gln Glu Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro 335 340 345	1481
45	aag ccc agc ttc tgg ccg aca gcc cag aac tcg ggg ggc tca gct tac Lys Pro Ser Phe Trp Pro Thr Ala Gln Asn Ser Gly Gly Ser Ala Tyr 350 355 360	1529
50	agt gag gag agg gat cgg cca tac ggc ctg gtg tcc att gac aca gtg Ser Glu Glu Arg Asp Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val 365 370 375	1577
55	act gtg cta gat gca gag ggg cca tgc acc tgg ccc tgc agc tgt gag	1625

5	Thr Val Leu Asp Ala Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu 380 385 390 395	
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15	cca ggc cta gag gac cca ctc ttg gat gca ggg acc aca gtc ctg tcc Pro Gly Leu Glu Asp Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser 415 420 425	1721
20	tgt ggc tgt gtc tca gct ggc agc cct ggg cta gga ggg ccc ctg gga Cys Gly Cys Val Ser Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly 430 435 440	1769
25	agc ctc ctg gac aga cta aag cca ccc ctt gca gat ggg gag gac tgg Ser Leu Leu Asp Arg Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp 445 450 455	1817
30	gct ggg gga ctg ccc tgg ggt ggc cgg tca cct gga ggg gtc tca gag Ala Gly Gly Leu Pro Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu 460 465 470 475	1865
35	agt gag gcg ggc tca ccc ctg gcc ggc ctg gat atg gac acg ttt gac Ser Glu Ala Gly Ser Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp 480 485 490	1913
40	agt ggc ttt gtg ggc tct gac tgc agc agc cct gtg gag tgt gac ttc Ser Gly Phe Val Gly Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe 495 500 505	1961
45	acc agc ccc ggg gac gaa gga ccc ccc cgg agc tac ctc cgc cag tgg Thr Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp 510 515 520	2009
50	gtg gtc att cct ccg cca ctt tcg agc cct gga ccc cag gcc agc taa Val Val Ile Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser 525 530 535	2057
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10	ctgatgttta cagtgtctgt gtgtgtgtc atatgtgtgt gtgtgcataat gcatgtgtgt	2237
15	gtgtgtgtgt gtcttaggtg cgcaatggca tgtccacgtg tgtgtgattt cacgtgcctg	2297
20	tgggcctggg ataatgcccataatgttccatgcatttaccatgcctgtgc atgtctggac	2357
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	Cys	Ser	Leu	His	Arg	Ser	Ala	His	Asn	Ala	Thr	His	Ala	Thr	Tyr	Thr	
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10	tgc	cac	atg	gat	gta	ttc	cac	ttc	atg	gcc	gac	gac	att	ttc	agt	gtc	288
	Cys	His	Met	Asp	Val	Phe	His	Phe	Met	Ala	Asp	Asp	Ile	Phe	Ser	Val	
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15	aac	atc	aca	gac	cag	tct	gac	aac	tac	tcc	cag	gag	tgt	ggc	agc	ttt	336
	Asn	Ile	Thr	Asp	Gln	Ser	Gly	Asn	Tyr	Ser	Gln	Glu	Cys	Gly	Ser	Phe	
																100	
																105	
																110	
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	Leu	Leu	Ala	Glu	Ser	Ile	Lys	Pro	Ala	Pro	Pro	Phe	Asn	Val	Thr	Val	
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																120	
																125	
25	acc	ttc	tca	gga	cag	tat	aat	atc	tcc	tgg	cgc	tca	gat	tac	gaa	gac	432
	Thr	Phe	Ser	Gly	Gln	Tyr	Asn	Ile	Ser	Trp	Arg	Ser	Asp	Tyr	Glu	Asp	
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																135	
																140	
30	cct	gcc	ttc	tac	atg	ctg	aag	ggc	aag	ctt	cag	tat	gag	ctg	cag	tac	480
	Pro	Ala	Phe	Tyr	Met	Leu	Lys	Gly	Lys	Leu	Gln	Tyr	Glu	Leu	Gln	Tyr	
																145	
																150	
																155	
																160	
35	agg	aac	cgg	gga	gac	ccc	tgg	gct	gtg	agt	ccg	agg	aga	aag	ctg	atc	528
	Arg	Asn	Arg	Gly	Asp	Pro	Trp	Ala	Val	Ser	Pro	Arg	Arg	Lys	Leu	Ile	
																165	
																170	
																175	
40	tca	gtg	gac	tca	aga	agt	gtc	tcc	ctc	ccc	ctg	gag	ttc	cgc	aaa		576
	Ser	Val	Asp	Ser	Arg	Ser	Val	Ser	Leu	Leu	Pro	Leu	Glu	Phe	Arg	Lys	
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																185	
																190	
45	gac	tcg	agc	tat	gag	ctg	cag	gtg	cg	gca	ggg	ccc	atg	cct	ggc	tcc	624
	Asp	Ser	Ser	Tyr	Glu	Leu	Gln	Val	Arg	Ala	Gly	Pro	Met	Pro	Gly	Ser	
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																200	
																205	
50	tcc	tac	cag	ggg	acc	tgg	agt	gaa	tgg	agt	gac	ccg	gtc	atc	ttt	cag	672
	Ser	Tyr	Gln	Gly	Thr	Trp	Ser	Glu	Trp	Ser	Asp	Pro	Val	Ile	Phe	Gln	

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	Thr Gln Ser Glu Thr Ala Trp Ile Ser Leu Val Thr Ala Leu His Leu			
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10	gtg ctg ggc ctc agc gcc gtc ctg ggc ctg ctg ctg agg tgg cag			768
	Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu Leu Arg Trp Gln			
	245	250	255	
15	ttt cct gca cac tac agg aga ctg agg cat gcc ctg tgg ccc tca ctt			816
	Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu			
	260	265	270	
20	cca gac ctg cac cgg gtc cta ggc cag tac ctt agg gac act gca gcc			864
	Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala			
	275	280	285	
25	ctg agc ccg ccc aag gcc aca gtc tca gat acc tgt gaa gaa gtg gaa			912
	Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys Glu Glu Val Glu			
	290	295	300	
30	ccc agc ctc ctt gaa atc ctc ccc aag tcc tca gag agg act cct ttg			960
	Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Arg Thr Pro Leu			
	305	310	315	320
35	ccc ctg tgt tcc tcc cag gcc cag atg gac tac cga aga ttg cag cct			1008
	Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg Arg Leu Gln Pro			
	325	330	335	
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	Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu			
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45	tca ggg tcc tgc tgt acc acc cac att gcc aac cat tcc tac cta cca			1104
	Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro			
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20
 Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
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 Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
 50 55 60

25
 Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
 65 70 75 80

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 Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
 85 90 95

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 Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
 100 105 110

40
 Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
 115 120 125

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 Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
 130 135 140

45
 Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
 145 150 155 160

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 Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
 165 170 175

Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
 180 185 190
 5 Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
 195 200 205
 10 Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
 210 215 220
 15 Thr Gln Ser Glu Thr Ala Trp Ile Ser Leu Val Thr Ala Leu His Leu
 225 230 235 240
 Val Leu Gly Leu Ser Ala Val Leu Gly Leu Leu Leu Leu Arg Trp Gln
 245 250 255
 20 Phe Pro Ala His Tyr Arg Arg Leu Arg His Ala Leu Trp Pro Ser Leu
 260 265 270
 25 Pro Asp Leu His Arg Val Leu Gly Gln Tyr Leu Arg Asp Thr Ala Ala
 275 280 285
 30 Leu Ser Pro Pro Lys Ala Thr Val Ser Asp Thr Cys Glu Glu Val Glu
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 Pro Ser Leu Leu Glu Ile Leu Pro Lys Ser Ser Glu Arg Thr Pro Leu
 305 310 315 320
 35 Pro Leu Cys Ser Ser Gln Ala Gln Met Asp Tyr Arg Arg Leu Gln Pro
 325 330 335
 Ser Cys Leu Gly Thr Met Pro Leu Ser Val Cys Pro Pro Met Ala Glu
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 40 Ser Gly Ser Cys Cys Thr Thr His Ile Ala Asn His Ser Tyr Leu Pro
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Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
20 25 3025 gtc atc tgc atc ctg gaa atg tgg aac ctc cac ccc agc acg ctc acc 144
Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 4530 ctt acc tgg caa gac cag tat gaa gag ctg aag gac gag gcc acc tcc 192
Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 6035 tgc agc ctc cac agg tcg gcc cac aat gcc acg cat gcc acc tac acc 240
Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 8040 tgc cac atg gat gta ttc cac ttc atg gcc gac gac att ttc agt gtc 288
Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
85 90 9545 aac atc aca gac cag tct ggc aac tac tcc cag gag tgt ggc agc ttt 336
Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
100 105 11050 ctc ctg gct gag agc atc aag ccg gct ccc cct ttc aac gtg act gtg 384
Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
115 120 125

55

5	acc ttc tca gga cag tat aat atc tcc tgg cgc tca gat tac gaa gac Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp 130 135 140	432
10	cct gcc ttc tac atg ctg aag ggc aag ctt cag tat gag ctg cag tac Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr 145 150 155 160	480
15	agg aac cgg gga gac ccc tgg gct gtg agt ccg agg aga aag ctg atc Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile 165 170 175	528
20	tca gtg gac tca aga agt gtc tcc ctc ccc ctg gag ttc cgc aaa Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys 180 185 190	576
25	gac tcg agc tat gag ctg cag gtg cgg gca ggg ccc atg cct ggc tcc Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser 195 200 205	624
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35	acc cag tca gag gag ccc aaa tct tgt gac aaa act cac aca tgc cca Thr Gln Ser Glu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro 225 230 235 240	720
40	ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe 245 250 255	768
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55	aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg	912

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro			
290	295	300	
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cg g gag gag cag tac aac agc acg tac cg g gt g gtc agc gtc ctc acc	960		
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr			
305	310	315	320
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gt c ct g cac cag gac tgg ct g aat ggc aag gag tac aag tgc aag gtc	1008		
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val			
325	330	335	
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tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc	1056		
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala			
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Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg			
355	360	365	
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gat gag ct g acc aag aac cag gtc agc ct g acc tgc ct g gtc aaa ggc	1152		
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly			
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Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro			
385	390	395	400
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gag aac aac tac aag acc acg cct ccc gt g ct g gac tcc gac ggc tcc	1248		
Glu Asn Asn Tyr Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser			
405	410	415	
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ttc ttc ctc tac agc aag ctc acc gt g gac aag agc agg tgg cag cag	1296		
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln			
420	425	430	
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ggg aac gtc ttc tca tgc tcc gt g at g cat gag gct ct g cac aac cac	1344		
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His			
435	440	445	
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455

460

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<400> 16

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20

Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
20 25 30

25

Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
35 40 45

30

Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
50 55 60Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
65 70 75 80

35

Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
85 90 95

40

Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe
100 105 110

45

Leu Leu Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
115 120 125Thr Phe Ser Gly Gln Tyr Asn Ile Ser Trp Arg Ser Asp Tyr Glu Asp
130 135 140

50

Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
145 150 155 160

55

Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
165 170 175

Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
 180 185 190
 5 Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
 195 200 205
 10 Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
 210 215 220
 15 Thr Gln Ser Glu Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
 225 230 235 240
 20 Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 245 250 255
 25 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 260 265 270
 30 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 275 280 285
 35 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 290 295 300
 40 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 305 310 315 320
 45 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 325 330 335
 50 Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 340 345 350
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 355 360 365
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 370 375 380
 55 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro

385 390 395 400

5 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 405 410 415

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 420 425 430

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 435 440 445

15 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
450 455 460

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40 ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc gat tac ctc cag acg 96
 Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
 20 25 30

45 gtc atc tgc atc ctg gaa atg tgg aac ctc cac ccc agc acg ctc acc 144
 Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
 35 40 45

50 ctt acc tgg caa gac cag tat gaa gag ctg aag gac gag gcc acc tcc 192
 Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
 50 55 60

5	tgc agc ctc cac agg tcg gcc cac aat gcc acg cat gcc acc tac acc Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr 65 70 75 80	240
10	tgc cac atg gat gta ttc cac ttc atg gcc gac gac att ttc agt gtc Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val 85 90 95	288
15	aac atc aca gac cag tct ggc aac tac tcc cag gag tgt ggc agc ttt Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe 100 105 110	336
20	ctc ctg gct gag agc aag tcc gag gag aaa gct gat ctc agt gga ctc Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala Asp Leu Ser Gly Leu 115 120 125	384
25	aag aag tgt ctc cct cct ccc cct gga gtt ccg caa aga ctc gag cta Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro Gln Arg Leu Glu Leu 130 135 140	432
30	agg gcg cgc cag gac tac aag gac gac gat gac aag acg cgt taa Arg Ala Arg Gln Asp Tyr Lys Asp Asp Asp Lys Thr Arg 145 150 155	477
35	<210> 18 <211> 158 <212> PRT <213> Homo sapiens	
40	<400> 18 Met Pro Arg Gly Trp Ala Ala Pro Leu Leu Leu Leu Leu Gln Gly 1 5 10 15	
45	Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr 20 25 30	
50	Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr 35 40 45	
	Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser	

	50	55	60	
5	Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr			
	65	70	75	80
10	Cys His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val			
	85	90		95
15	Asn Ile Thr Asp Gln Ser Gly Asn Tyr Ser Gln Glu Cys Gly Ser Phe			
	100	105		110
20	Leu Leu Ala Glu Ser Lys Ser Glu Glu Lys Ala Asp Leu Ser Gly Leu			
	115	120	125	
25	Lys Lys Cys Leu Pro Pro Pro Gly Val Pro Gln Arg Leu Glu Leu			
	130	135	140	
30	Arg Ala Arg Gln Asp Tyr Lys Asp Asp Asp Asp Lys Thr Arg			
	145	150	155	
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	<212> PRT			
	<213> Mus musculus			
40	<400> 19			
	Met Pro Arg Gly Trp Ala Ala Ser Leu Leu Leu Leu Gln Gly			
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45	Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr			
	20	25	30	
50	Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr			
	35	40	45	
55	Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser			
	50	55	60	
60	Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr			
	65	70	75	80

Ser His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
 85 90 95

5 Asn Ile Thr Asp Gln Ser Gly Asn Tyr Phe Gln Glu Cys Gly Ser Phe
 100 105 110

10 Leu Arg Ala Glu Ser Lys Ser Glu Glu Lys Ala Asp Leu Ser Gly Leu
 115 120 125

15 Lys Lys Cys Leu Pro Pro Pro Gly Val Pro Gln Arg Leu Glu Leu
 130 135 140

20 <210> 20

<211> 1960

<212> DNA

<213> *Mus musculus*

25 <400> 20

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tgtctgcggc ccgtgcattcc ctgctgcggc cgcctggta cttcccttgcc gtcttttcc 180

tctgtctgtct gtcttgtggg acacctgcct ggaggccca gtcgttgc tca 240

aggctttatg acagcctgat tggtgactcg ggctgggtgt ggatttcac cccaggcctc 300

tgcctgtttt ctca gacccat ctcggta cccacgtg aacccatgt ccacccca 360

aagcccatca gactgcccc agcacacgga atggatttct gagaagaag ccgaaacaga 420

45 aggccctgtgg gagtcagg atg ccg cgt ggc tgg gcc tcc ttg ctc ctg 471

Met Pro Arg Gly Trp Ala Ala Ser Leu Leu Leu

1 5 10

50 ctg ctg ctc cag gga ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc 519
 Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr

15 20 25

55

5	gat tac ctc cag acg gtc atc tgc atc ctg gaa atg tgg aac ctc cac Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His 30 35 40	567
10	ccc agc acg ctc acc ctt acc tgg caa gac cag tat gaa gag ctg aag Pro Ser Thr Leu Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys 45 50 55	615
15	gac gag gcc acc tcc tgc agc ctc cac agg tcg gcc cac aat gcc acg Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr 60 65 70 75	663
20	cat gcc acc tac acc agc cac atg gat gta ttc cac ttc atg gcc gac His Ala Thr Tyr Thr Ser His Met Asp Val Phe His Phe Met Ala Asp 80 85 90	711
25	gac att ttc agt gtc aac atc aca gac cag tct ggc aac tac ttc cag Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Phe Gln 95 100 105	759
30	gag tgt ggc agc ttt ctc cgg gct gag agc aag tcc gag gag aaa gct Glu Cys Gly Ser Phe Leu Arg Ala Glu Ser Lys Ser Glu Glu Lys Ala 110 115 120	807
35	gat ctc agt gga ctc aag aag tgt ctc cct cct ccc cct gga gtt ccg Asp Leu Ser Gly Leu Lys Lys Cys Leu Pro Pro Pro Pro Gly Val Pro 125 130 135	855
40	caa aga ctc gag cta tgagctgcag gtgcggcag ggccatgcc tggtctcc Gln Arg Leu Glu Leu 140	910
45	taccagggga cctggagtga atggagtgac ccggtcatct ttcatgacca gtcagaggag 970	
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40 <210> 21

<211> 538

<212> PRT

<213> *Mus musculus*

45 <400> 21

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50 Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr Asp Tyr Leu Gln Thr
 20 25 30

Val Ile Cys Ile Leu Glu Met Trp Asn Leu His Pro Ser Thr Leu Thr
 35 40 45

5 Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys Asp Glu Ala Thr Ser
 50 55 60

10 Cys Ser Leu His Arg Ser Ala His Asn Ala Thr His Ala Thr Tyr Thr
 65 70 75 80

15 Ser His Met Asp Val Phe His Phe Met Ala Asp Asp Ile Phe Ser Val
 85 90 95

Asn Ile Thr Asp Gln Ser Gly Asn Tyr Phe Gln Glu Cys Gly Ser Phe
 100 105 110

20 Leu Arg Ala Glu Ser Ile Lys Pro Ala Pro Pro Phe Asn Val Thr Val
 115 120 125

25 Thr Phe Ser Gly Gln Tyr Asn Ile Ser Arg Arg Ser Asp Tyr Glu Asp
 130 135 140

30 Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln Tyr Glu Leu Gln Tyr
 145 150 155 160

Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro Arg Arg Lys Leu Ile
 165 170 175

35 Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro Leu Glu Phe Arg Lys
 180 185 190

40 Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly Pro Met Pro Gly Ser
 195 200 205

45 Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp Pro Val Ile Phe Gln
 210 215 220

Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn Pro His Leu Leu Leu
 225 230 235 240

50 Leu Leu Leu Val Ile Val Phe Ile Pro Ala Phe Trp Ser Leu Lys
 245 250 255

Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile Trp Ala Val Pro Ser	260	265	270	
Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly Cys Ser Gly Asp Phe	275	280	285	
Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser Ser Leu Glu Leu Gly	290	295	300	
Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu Val Tyr Ser Cys His	305	310	315	320
Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu Thr Glu Leu Gln Glu	325	330	335	
Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro Lys Pro Ser Phe Trp	340	345	350	
Pro Thr Ala Gln Asn Ser Gly Gly Ser Ala Tyr Ser Glu Glu Arg Asp	355	360	365	
Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val Thr Val Leu Asp Ala	370	375	380	
Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu Asp Asp Gly Tyr Pro	385	390	395	400
Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser Pro Gly Leu Glu Asp	405	410	415	
Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser Cys Gly Cys Val Ser	420	425	430	
Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly Ser Leu Leu Asp Arg	435	440	445	
Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp Ala Gly Gly Leu Pro	450	455	460	
Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu Ser Glu Ala Gly Ser				

465 470 475 480

5 Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp Ser Gly Phe Val Cys
485 490 495

10 Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe Thr Ser Pro Gly Asp
500 505 510

15 Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp Val Val Ile Pro Pro
515 520 525

20 Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
530 535

25 <210> 22
<211> 2115
<212> DNA
<213> *Mus musculus*

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40 tgtctgcggc ccgtgcattcc ctgctgcggc cgcctggta cttccttgcc gtctttcc 180

45 tctgtctgct gctctgtggg acacctgct ggaggcccctgcccgtca tcagagtgc 240

50 aggtcttatg acagcctgat tggtaactcg ggctgggtgt ggattctcac cccaggec 300

55 tgcctgctt ctcagaccct catcggtca cccacgctg aacccagctg ccacccccc 360

60 aagcccatca gactgcccc agcacacgga atggatttct gagaaagaag ccgaaacaga 420

65 aggcccgtgg gagtcagc atg ccg cgt ggc tgg gcc tcc ttg ctc ctg 471
Met Pro Arg Gly Trp Ala Ala Ser Leu Leu Leu

1 5 10

70 ctg ctg ctc cag gga ggc tgg ggc tgc ccc gac ctc gtc tgc tac acc 519
Leu Leu Leu Gln Gly Gly Trp Gly Cys Pro Asp Leu Val Cys Tyr Thr

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5	gat tac ctc cag acg gtc atc tgc atc ctg gaa atg tgg aac ctc cac			567
	Asp Tyr Leu Gln Thr Val Ile Cys Ile Leu Glu Met Trp Asn Leu His			
	30	35	40	
10	ccc agc acg ctc acc ctt acc tgg caa gac cag tat gaa gag ctg aag			615
	Pro Ser Thr Leu Thr Trp Gln Asp Gln Tyr Glu Glu Leu Lys			
	45	50	55	
15	gac gag gcc acc tcc tgc agc ctc cac agg tgc gcc cac aat gcc acg			663
	Asp Glu Ala Thr Ser Cys Ser Leu His Arg Ser Ala His Asn Ala Thr			
	60	65	70	75
20	cat gcc acc tac acc agc cac atg gat gta ttc cac ttc atg gcc gac			711
	His Ala Thr Tyr Thr Ser His Met Asp Val Phe His Phe Met Ala Asp			
	80	85	90	
25	gac att ttc agt gtc aac atc aca gac cag tct ggc aac tac ttc cag			759
	Asp Ile Phe Ser Val Asn Ile Thr Asp Gln Ser Gly Asn Tyr Phe Gln			
	95	100	105	
30	gag tgt ggc agc ttt ctc cgg gct gag agc atc aag ccg gct ccc cct			807
	Glu Cys Gly Ser Phe Leu Arg Ala Glu Ser Ile Lys Pro Ala Pro Pro			
	110	115	120	
35	ttc aac gtg act gtg acc ttc tca gga cag tat aat atc tcc agg cgc			855
	Phe Asn Val Thr Val Thr Phe Ser Gly Gln Tyr Asn Ile Ser Arg Arg			
	125	130	135	
40	tca gat tac gaa gac cct gcc ttc tac atg ctg aag ggc aag ctt cag			903
	Ser Asp Tyr Glu Asp Pro Ala Phe Tyr Met Leu Lys Gly Lys Leu Gln			
	140	145	150	155
45	tat gag ctg cag tac agg aac cgg gga gac ccc tgg gct gtg agt ccg			951
	Tyr Glu Leu Gln Tyr Arg Asn Arg Gly Asp Pro Trp Ala Val Ser Pro			
	160	165	170	
50	agg aga aag ctg atc tca gtg gac tca aga agt gtc tcc ctc ctc ccc			999
	Arg Arg Lys Leu Ile Ser Val Asp Ser Arg Ser Val Ser Leu Leu Pro			
	175	180	185	

55

5	ctg gag ttc cgc aaa gac tcg agc tat gag ctg cag gtg egg gca ggg Leu Glu Phe Arg Lys Asp Ser Ser Tyr Glu Leu Gln Val Arg Ala Gly 190 195 200	1047
10	ccc atg cct ggc tcc tcc tac cag ggg acc tgg agt gaa tgg agt gac Pro Met Pro Gly Ser Ser Tyr Gln Gly Thr Trp Ser Glu Trp Ser Asp 205 210 215	1095
15	ccg gtc atc ttt cag acc cag tca gag gag tta aag gaa ggc tgg aac Pro Val Ile Phe Gln Thr Gln Ser Glu Glu Leu Lys Glu Gly Trp Asn 220 225 230 235	1143
20	cct cac ctg ctg ctt ctc ctc ctg ctt gtc ata gtc ttc att cct gcc Pro His Leu Leu Leu Leu Leu Leu Val Ile Val Phe Ile Pro Ala 240 245 250	1191
25	ttc tgg agc ctg aag acc cat cca ttg tgg agg cta tgg aag aag ata Phe Trp Ser Leu Lys Thr His Pro Leu Trp Arg Leu Trp Lys Lys Ile 255 260 265	1239
30	tgg gcc gtc ccc agc cct gag cgg ttc ttc atg ccc ctg tac aag ggc Trp Ala Val Pro Ser Pro Glu Arg Phe Phe Met Pro Leu Tyr Lys Gly 270 275 280	1287
35	tgc agc gga gac ttc aag aaa tgg gtg ggt gca ccc ttc act ggc tcc Cys Ser Gly Asp Phe Lys Lys Trp Val Gly Ala Pro Phe Thr Gly Ser 285 290 295	1335
40	agc ctg gag ctg gga ccc tgg agc cca gag gtg ccc tcc acc ctg gag Ser Leu Glu Leu Gly Pro Trp Ser Pro Glu Val Pro Ser Thr Leu Glu 300 305 310 315	1383
45	gtg tac agc tgc cac cca cca cgg agc cgg gcc aag agg ctg cag ctc Val Tyr Ser Cys His Pro Pro Arg Ser Pro Ala Lys Arg Leu Gln Leu 320 325 330	1431
50	acg gag cta caa gaa cca gca gag ctg gtg gag tct gac ggt gtg ccc Thr Glu Leu Gln Glu Pro Ala Glu Leu Val Glu Ser Asp Gly Val Pro 335 340 345	1479

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10	agt gag gag agg gat cgg cca tac ggc ctg gtg tcc att gac aca gtg Ser Glu Glu Arg Asp Arg Pro Tyr Gly Leu Val Ser Ile Asp Thr Val 365 370 375	1575
15	act gtg cta gat gca gag ggg cca tgc acc tgg ccc tgc agc tgt gag Thr Val Leu Asp Ala Glu Gly Pro Cys Thr Trp Pro Cys Ser Cys Glu 380 385 390 395	1623
20	gat gac ggc tac cca gcc ctg gac ctg gat gct ggc ctg gag ccc agc Asp Asp Gly Tyr Pro Ala Leu Asp Leu Asp Ala Gly Leu Glu Pro Ser 400 405 410	1671
25	cca ggc cta gag gac cca ctc ttg gat gca ggg acc aca gtc ctg tcc Pro Gly Leu Glu Asp Pro Leu Leu Asp Ala Gly Thr Thr Val Leu Ser 415 420 425	1719
30	tgt ggc tgt gtc tca gct ggc agc cct ggg cta gga ggg ccc ctg gga Cys Gly Cys Val Ser Ala Gly Ser Pro Gly Leu Gly Gly Pro Leu Gly 430 435 440	1767
35	agc ctc ctg gac aga cta aag cca ccc ctt gca gat ggg gag gac tgg Ser Leu Leu Asp Arg Leu Lys Pro Pro Leu Ala Asp Gly Glu Asp Trp 445 450 455	1815
40	gct ggg gga ctg ccc tgg ggt ggc cgg tca cct gga ggg gtc tca gag Ala Gly Gly Leu Pro Trp Gly Gly Arg Ser Pro Gly Gly Val Ser Glu 460 465 470 475	1863
45	agt gag gcg ggc tca ccc ctg gcc ggc ctg gat atg gac acg ttt gac Ser Glu Ala Gly Ser Pro Leu Ala Gly Leu Asp Met Asp Thr Phe Asp 480 485 490	1911
50	agt ggc ttt gtg tgc tct gac tgc agc agc cct gtg gag tgt gac ttc Ser Gly Phe Val Cys Ser Asp Cys Ser Ser Pro Val Glu Cys Asp Phe 495 500 505	1959
55	acc agc ccc ggg gac gaa gga ccc ccc cgg agc tac ctc cgc cag tgg	2007

Thr Ser Pro Gly Asp Glu Gly Pro Pro Arg Ser Tyr Leu Arg Gln Trp
 510 515 520

5 gtg gtc att cct ccg cca ctt tcg agc cct gga ccc cag gcc agc 2052
 Val Val Ile Pro Pro Pro Leu Ser Ser Pro Gly Pro Gln Ala Ser
 525 530 535

10 taatgaggct gactggatgt ccagagctgg ccaggccact gggccctgag ccagaaaaaa 2112

aaa 2115

15 <210> 23

<211> 411

20 <212> DNA

<213> Homo sapiens

25 <220>

<221> 3' UTR

<222> (1)..(411)

30 <400> 23

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35 agcctgtatgtt tacagtgtc tgtgtgtgtg tgcataatgtg tgtgtgtgca tatgcataatgtg 180

tgtgtgtgtg tgtgtcttag gtgcgcagtgc gcatgtccac gtgtgtgtga ttgcacgtgc 240

40 ctgtggccct gggataatgc ccatggtaact ccatgcattc acctggccctg tgcataatgtg 300

gactcacgga gctcacccat gtgcacaatgtg gtgcacagta aacgtgtttg tggtaacag 360

45 aaaaaaaaaaaa aaaaaaaaaaaa aaaaaaaaaaaa aaaaaaaaaaaa aaaaaaaaaaaa a 411

50 <210> 24

<211> 877

<212> DNA

<213> Homo sapiens

<220>
<221> 3' UTR
<222> (1)..(877)

<400> 24

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tgtgtgtgtg tgtgtgtctt aggtgcgcag tggcatgtcc acgtgtgtgt gtgattgcac 240

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ctccgggct ttgtggatc agggcattgc ctgtgactga ggccggagccc agccctccag 480

cgctcgctc caggagctgc aagaagtcca tattgttctt tatcacctgc caacaggaag 540

cggaaagggraa tggagtggc ccatggtgac ctcgggaaatg gcaattttt gggcgggcccc 600

tggacgaagg tctgaatccc gactctgata ccttctggct gtgctacctg agccaaatcg 660

cctccctctt ctgggctaga gtttccttat ccagacatgt gggaaaggcat gacacacac 720

gggaaattg gcgatgtcac ccgtgtacgg tacgcagccc agagcagacc ctcaataaac 780

gtcagcttcc ttcccttctgc ggccagagcc gaggcgccggcg ggggtgagaa catcaatcgt 840

cagcgacaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa

877

<210> 25

<211> 2791

<212> DNA

<213> *Homo sapiens*

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 <221> 3' UTR
 <222> (1)..(2791)

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 agcctgatgt ttacagtgtc tgtgtgtgtg tgtgcataatg tgtgtgtgtg catatgcac 180
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 tgtgtgtgtg tgtgtgtctt aggtgcgcag tggcatgtcc aegtgtgtgt gtgattgcac 240
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Claims

1. A protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.
2. A protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.
3. A protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.
4. A protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7.
5. A protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19.
6. A protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21, or a protein comprising a modified amino acid sequence of said amino acid sequence in which one or more amino acids have been deleted, added and/or substituted with another amino acid, and being functionally equivalent to the protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21.
7. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 2, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 361st amino acid Ser of SEQ ID NO: 1.
8. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 4, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 3.
9. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 6, which is functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 237th amino acid Ser of SEQ ID NO: 5.
10. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 8, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 7.
11. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 20, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 144th amino acid Leu of SEQ ID NO: 19.

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12. A protein encoded by a DNA hybridizing to a DNA comprising the nucleotide sequence of SEQ ID NO: 22, said protein being functionally equivalent to a protein comprising the amino acid sequence from the 1st amino acid Met to the 538th amino acid Ser of SEQ ID NO: 21.

5 13. A fusion protein comprising the protein of any one of claims 1 to 12 and another peptide or polypeptide.

14. A DNA encoding the protein of any one of claims 1 to 13.

15. A vector comprising the DNA of claim 14.

10 16. A transformant harboring the DNA of claim 14 in an expressible manner.

17. A method of producing the protein of any one of claims 1 to 13, comprising the step of culturing the transformant of claim 16.

15 18. A method of screening a compound that binds to the protein of any one of claims 1 to 13 comprising the steps of:

- (a) contacting a test sample with the protein of any one of claims 1 to 13; and
- (b) selecting a compound that comprises an activity to bind to the protein of any one of claims 1 to 13.

20 19. An antibody that specifically binds to the protein of any one of claims 1 to 12.

20 20. A method of detecting or measuring the protein of any one of claims 1 to 13 comprising the steps of contacting a test sample presumed to contain said protein with the antibody of claim 19, and detecting or measuring the formation of the immune complex between the antibody and the protein.

25 21. A DNA specifically hybridizing to a DNA comprising a nucleotide sequence of any one of SEQ ID NOs: 2, 4, 6, 8, 20, and 22 to 27 comprising at least 15 nucleotides, and comprising at least 15 nucleotides.

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55

Figure 1

NR8*	40862	<u>SLLP</u> <u>LEFRK</u> <u>DSSY</u> <u>ELQVR</u> <u>AGPMP</u> <u>GSSY</u> <u>QGT</u> <u>TWE</u> <u>SDPV</u> <u>IFQT</u> <u>QSE</u> <u>GRCE</u> <u>AGMD</u> <u>TPLL</u>	41032
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h0BR	292	<u>SLL</u> <u>VD</u> <u>SIL</u> <u>PG</u> <u>GSSY</u> <u>EY</u> <u>QVR</u> <u>GK</u> <u>R</u> <u>LDG</u> <u>P</u> <u>--G</u> <u>IWS</u> <u>DW</u> <u>S</u> <u>PRV</u> <u>FTIQ</u>	331
hIL2Rb	201	<u>D</u> <u>T</u> <u>Q</u> <u>Y</u> <u>E</u> <u>F</u> <u>Q</u> <u>V</u> <u>R</u> <u>V</u> <u>K</u> <u>P</u> <u>LQ</u> <u>G</u> <u>E</u> <u>F</u> <u>T</u> <u>--T</u> <u>W</u> <u>S</u> <u>P</u> <u>Q</u> <u>L</u> <u>A</u> <u>F</u> <u>R</u> <u>T</u> <u>K</u>	232
hIL7R	189	<u>T</u> <u>L</u> <u>Q</u> <u>R</u> <u>K</u> <u>L</u> <u>Q</u> <u>A</u> <u>AM</u> <u>E</u> <u>I</u> <u>K</u> <u>V</u> <u>R</u> <u>S</u> <u>--I</u> <u>D</u> <u>D</u> <u>H</u> <u>F</u> <u>K</u> <u>G</u> <u>F</u> <u>W</u> <u>S</u> <u>E</u> <u>W</u> <u>S</u> <u>P</u> <u>Y</u> <u>Y</u> <u>F</u> <u>R</u> <u>T</u> <u>P</u> <u>E</u> <u>I</u> <u>N</u> <u>S</u> <u>S</u> <u>G</u> <u>E</u> <u>M</u> <u>D</u> <u>P</u> <u>I</u> <u>L</u> <u>L</u>	243
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mIL3Rb	200	<u>N</u> <u>LE</u> <u>P</u> <u>K</u> <u>L</u> <u>F</u> <u>L</u> <u>P</u> <u>N</u> <u>S</u> <u>I</u> <u>Y</u> <u>A</u> <u>R</u> <u>V</u> <u>T</u> <u>R</u> <u>L</u> <u>S</u> <u>A</u> <u>G</u> <u>S</u> <u>S</u> <u>L</u> <u>S</u> <u>G</u> <u>R</u> <u>P</u> <u>S</u> <u>R</u> <u>W</u> <u>S</u> <u>P</u> <u>E</u> <u>Y</u> <u>H</u> <u>W</u> <u>D</u> <u>S</u> <u>Q</u>	242
	404	<u>Q</u> <u>LE</u> <u>P</u> <u>D</u> <u>I</u> <u>S</u> <u>C</u> <u>A</u> <u>R</u> <u>V</u> <u>K</u> <u>P</u> <u>I</u> <u>--S</u> <u>D</u> <u>Y</u> <u>D</u> <u>G</u> <u>I</u> <u>W</u> <u>S</u> <u>N</u> <u>E</u> <u>Y</u> <u>T</u> <u>W</u> <u>T</u> <u>I</u>	438
hIL5Ra	302	<u>S</u> <u>K</u> <u>Y</u> <u>D</u> <u>V</u> <u>Q</u> <u>V</u> <u>R</u> <u>A</u> <u>V</u> <u>S</u> <u>M</u> <u>C</u> <u>R</u> <u>E</u> <u>A</u> <u>G</u> <u>I</u> <u>W</u> <u>S</u> <u>E</u> <u>W</u> <u>S</u> <u>Q</u> <u>P</u> <u>I</u>	329
hIL9R	241	<u>Y</u> <u>T</u> <u>G</u> <u>Q</u> <u>W</u> <u>S</u> <u>E</u> <u>W</u> <u>S</u> <u>Q</u> <u>P</u> <u>V</u> <u>C</u> <u>F</u> <u>Q</u>	255
hEP0R	211	<u>R</u> <u>G</u> <u>R</u> <u>T</u> <u>R</u> <u>T</u> <u>F</u> <u>A</u> <u>V</u> <u>R</u> <u>A</u> <u>R</u> <u>-M</u> <u>A</u> <u>E</u> <u>P</u> <u>S</u> <u>F</u> <u>G</u> <u>F</u> <u>W</u> <u>S</u> <u>A</u> <u>S</u> <u>P</u> <u>V</u> <u>S</u> <u>L</u> <u>T</u> <u>P</u> <u>S</u> <u>D</u>	247
hIL2Rr	209	<u>S</u> <u>L</u> <u>P</u> <u>S</u> <u>V</u> <u>D</u> <u>G</u> <u>Q</u> <u>K</u> <u>R</u> <u>T</u> <u>F</u> <u>V</u> <u>R</u> <u>S</u> <u>R</u> <u>F</u> <u>N</u> <u>P</u> <u>L</u> <u>G</u> <u>S</u> <u>A</u> <u>Q</u> <u>H</u> <u>--W</u> <u>S</u> <u>E</u> <u>W</u> <u>S</u> <u>H</u> <u>P</u> <u>I</u>	244
hIL12R	197	<u>L</u> <u>C</u> <u>P</u> <u>L</u> <u>M</u> <u>N</u> <u>V</u> <u>A</u> <u>Q</u> <u>E</u> <u>F</u> <u>Q</u> <u>L</u> <u>R</u> <u>R</u> <u>Q</u> <u>L</u> <u>G</u> <u>S</u> <u>G</u> <u>S</u> <u>S</u> <u>--W</u> <u>S</u> <u>K</u> <u>W</u> <u>S</u> <u>S</u> <u>P</u> <u>V</u>	229
hIL12Rb	282	<u>L</u> <u>D</u> <u>L</u> <u>K</u> <u>P</u> <u>F</u> <u>T</u> <u>E</u> <u>Y</u> <u>E</u> <u>F</u> <u>Q</u> <u>I</u> <u>S</u> <u>S</u> <u>K</u> <u>L</u> <u>--H</u> <u>L</u> <u>Y</u> <u>K</u> <u>G</u> <u>S</u> <u>M</u> <u>D</u> <u>W</u> <u>S</u> <u>E</u> <u>S</u> <u>L</u> <u>R</u> <u>A</u> <u>Q</u> <u>T</u> <u>P</u> <u>E</u> <u>E</u>	319

Figure 2

[Query: 39181-39360]

NR8	39233	<u>HQVKPAPPFN</u> — <u>VTVTSGQYNISWRS</u> — <u>DYEDP</u> — <u>AFYMLKGKLQY</u> 39355
hIL6Ra	214	<u>LQPDPPANI</u> — <u>TVTAVAR</u> — <u>NPRWLSVTWQDPHSWNSSFYRLRFELRY</u> 257
hgp130	218	<u>YKVKPNNPHNL</u> — <u>SVINSEELSSILKLTWT</u> — <u>NPSIKSV</u> — <u>IILKYNIQY</u> 261
r0BRb	234	<u>VKPDPLGLRMEVTDDGNLKISWDS</u> — <u>QTKAP</u> 263

[Query: 42301-42480]

NR8	42307	<u>VPSPERFFMPLYKGCSGDFK</u> 42366
mIL9R	305	<u>IPSPEAFFHPLYSVYHGDFQ</u> 324
hIL9R	305	<u>VPSPAMFFQPLYSVHNGNFO</u> 324

Figure 3

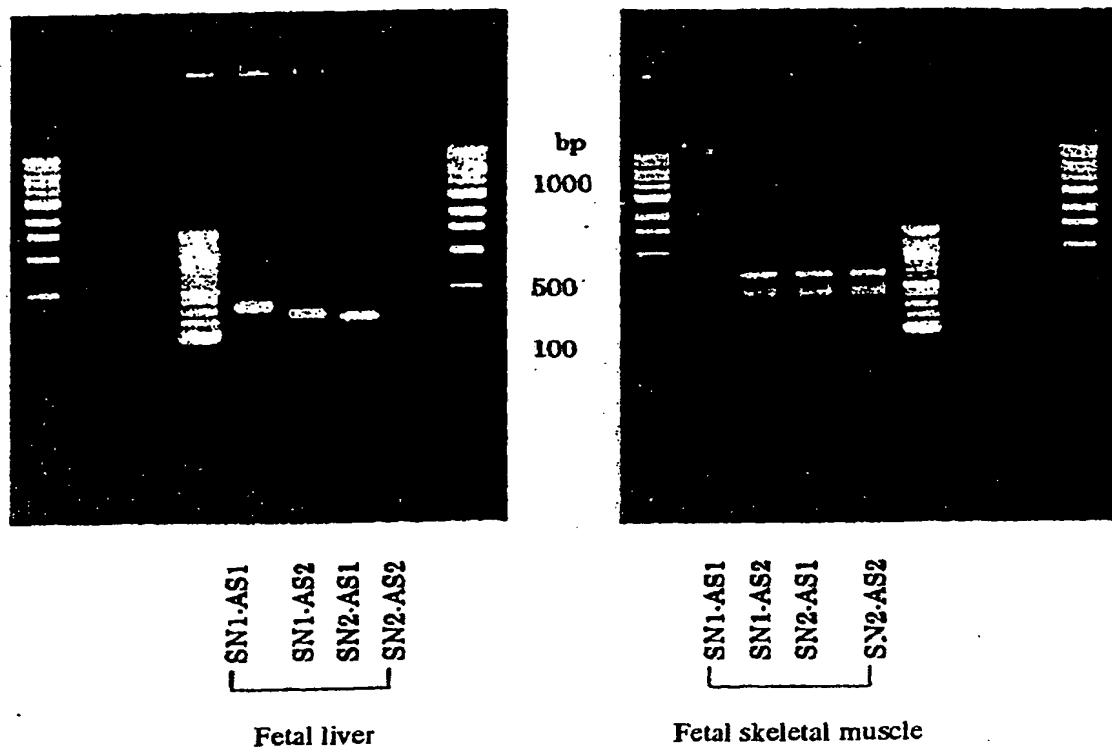


Figure 4

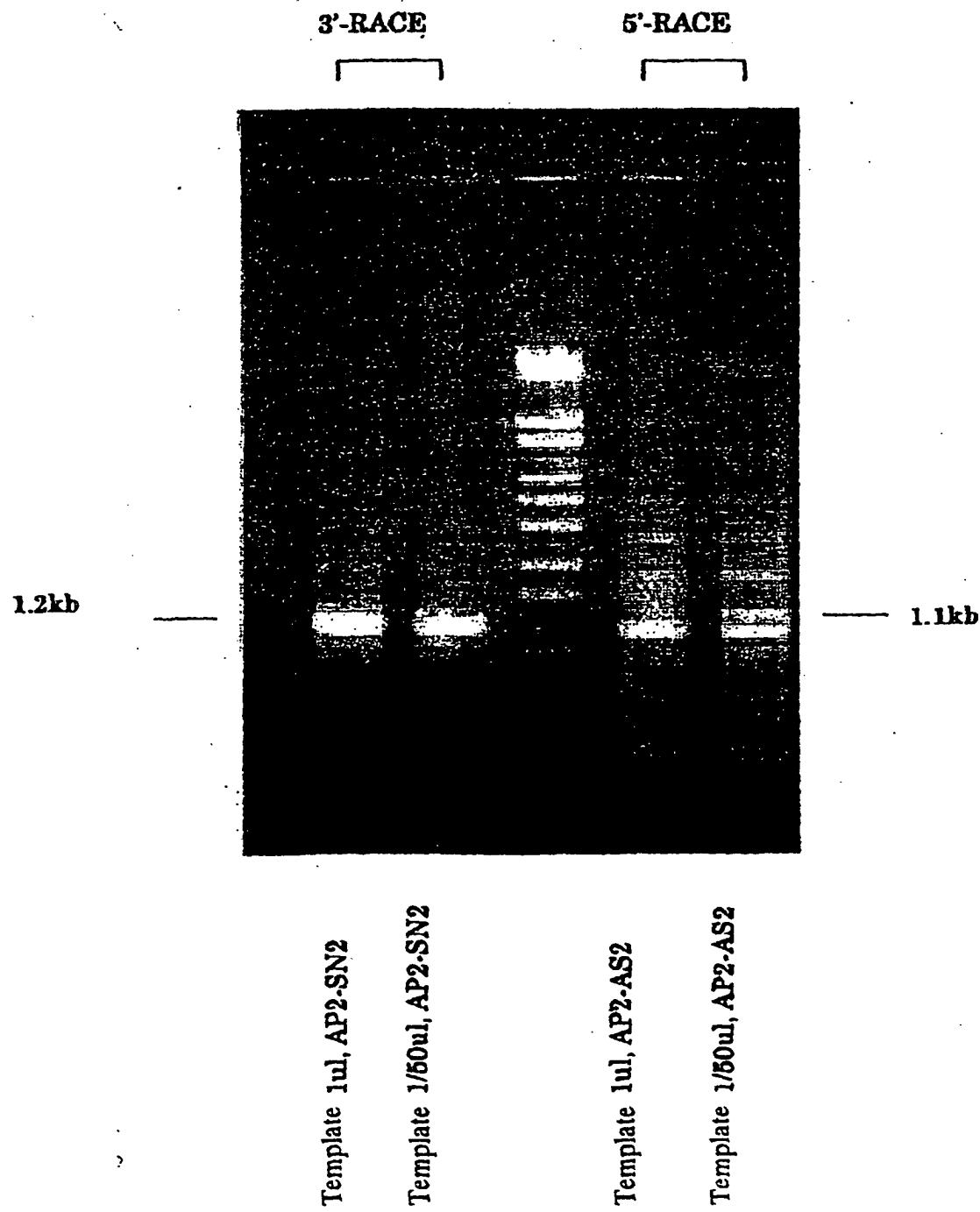


Figure 5

10 20 30 40 50 60 70 80
 GGCAGCCAGCGGCCTCAGACAGACCCACTGGCGTCTCTGCTGAGTGACCGTAAGCTGGCGTCTGGCCCTCTGCCTGC
 90 100 110 120 130 140 150 160
 CTCTCCCTGAGTGTGGCTGACAGCCACGCCAGCTGTCTGCTGCTGCGGCCGTGCATCCCTGCTGCCGCCGCTGGT
 170 180 190 200 210 220 230 240
 ACCTTCCTTGCCGTCTTCCCTGTCTGCTGCTGTGGACACCTGCCTGGAGGCCAGCTGCCGTATCAGACTG
 250 260 270 280 290 300 310 320
 ACAGGTCTTATGACAGCCTGATTGGTACTCGGGCTGGGTGTGGATTCTCACCCCAGGCCCTGCTGCTGCTTCAGACC
 330 340 350 360 370 380 390 400
 CTCATCTGTCACCCCAACGCTGAACCCAGCTGCCACCCCCAGAACGCCATCAGACTGCCAACACGGAAATGGATT
 410 420 430 440 450 460 470 480
 CTGAGAAAAGGCCAACAGAAGGCCGTGGGAGTCAGCATGCCCGTGGCTGGGCCGCCCTTGCTCCTGCTGCTGC
 M P R G W A A P L L L L L
 490 500 510 520 530 540 550 560
 TCCAGGGAGGCTGGGCTGCCCGACCTCGTCTGCTACACCGATTACCTCCAGACGGTCATCTGCATCCTGAAATGTGG
 Q G G W G C P D L V C Y T D Y L Q T V I C I L E M W
 570 580 590 600 610 620 630 640
 AACCTCCACCCAGCACGCTCACCTAACCTGGCAAGACCAAGTATGAAGAGCTGAAGGACGAGGCCACCTCCTGCAGCCT
 N L H P S T L T L T W Q D O Y E E L K D E A T S C S L
 650 660 670 680 690 700 710 720
 CCACAGGTGGCCACAATGCCACCGATGCCACCTACACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATT
 H R S A H N A T H A T Y T C H M D V F H F M A D D I F
 730 740 750 760 770 780 790 800
 TCA GTGTCAACATCACAGACCAAGTCTGGCAACTACTCCAGGAGTGTGGCAGCTTCTGGCTGAGAGCATCAAGCCG
 S V N I T D Q S G N Y S Q E C G S F L L A E S I K P
 810 820 830 840 850 860 870 880
 GCTCCCCCTTCAACGTGACTGTGACCTCTCAGGACAGTATAATATCTCCTGGCGTCAGATTACGAAGACCCCTGCCCT
 A P P F N V T V T F S G Q Y N I S W R S O Y E D P A F
 890 900 910 920 930 940 950 960
 CTACATGCTGAAGGGCAAGCTTCAGTATGAGCTGAGTACAGGAACCGGGAGACCCCTGGCTGTGAGTCCGAGGAGAA
 Y M L K G K L Q Y E L O Y R N R G D P W A V S P R R K
 970 980 990 1000 1010 1020 1030 1040
 AGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCTCCCCCTGGAGTTCCGAAAGACTCGAGCTATGAGCTGCAGGTG
 L I S V D S R S V S L L P L E F R K D S S Y E L Q V
 1050 1060 1070 1080 1090 1100 1110 1120
 CGGGCAGGGCCCATGCCCTGGCTCTCCTACCAAGGGACTGGAGTGAATGGAGTGACCCGGTCATCTTCAGACCCAGTC
 R A G P M P G S S Y Q G T W S E W S D P V I F Q T Q S

Figure 6

1130 1140 1150 1160 1170 1180 1190 1200
 AGAGGAGTTAAGGAAGGCTGGAACCCCTCACCTGCTGCTTCTCCTCCTGCTTGTCAAGTCATAGTCCTTCATTCCCTGCCCTCTGGA
 E E L K E G W N P H L L L L L L V I V F I P A F W S
 1210 1220 1230 1240 1250 1260 1270 1280
 GCCTGAAGACCCATCCATTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGCCCTG
 L K T H . P L W R L W K K I W A V P S P E R F F M P L
 1290 1300 1310 1320 1330 1340 1350 1360
 TACAAGGGCTGCAGCGGAGACTTCAAGAAATGGGTGGGTGCACCCCTTCACTGGCTCCAGCCTGGAGCTGGGACCCCTGGAG
 Y K G C S G D F K K W V G A P F T G S S L E L G P W S
 1370 1380 1390 1400 1410 1420 1430 1440
 CCCAGAGGTGCCCTCCACCCCTGGAGGTGTACAGCTGCCACCCACCCAGCAGGCCCTGTGGAGTGTGACTTCACCAAGCCCCG
 P E V P S T L E V Y S C H P P S S P V E C D F T S P G
 1450 1460 1470 1480 1490 1500 1510 1520
 GGGACGAAGGACCCCCCGGAGCTACCTCCGCCAGTGGGTGGTCAATTCCCTCCGCCACTTTCGAGGCCCTGGACCCAGGCC
 D E G P P R S Y L R Q W V V I P P P L S S P G P Q A
 1530 1540 1550 1560 1570 1580 1590 1600
 AGCTAATGAGGCTGACTGGATGTCCAGAGCTGGCCAGGCCACTGGGCCCTGAGCCAGAGACAAGGTACACCTGGGCTGTGA
 S * *
 1610 1620 1630 1640 1650 1660 1670 1680
 TGTGAAGACACCTGCAGCTTGGTCTCCTGGATGGGCCCTTGAGCCTGATGTTACAGTGTCTGTGTGTGCATAT
 1690 1700 1710 1720 1730 1740 1750 1760
 GTGTGTGTGCATATGCATGTGTGTGTGTGTCTAGGTGCCAGTGGCATGTCCACGTGTGTGTGATTGCACG
 1770 1780 1790 1800 1810 1820 1830 1840
 TGCCCTGGGCCCTGGATAATGCCCATGGTACTCCATGCATTCAACCTGCCCTGTGCATGTCTGGACTCACGGAGCTCACC
 1850 1860 1870 1880 1890 1900 1910 1920
 CATGTGCACAAGTGTGCACAGTAAACGTGTTGTGGTCAACAGAAAAAAA
 1930
 AAAAAAAA

Figure 7

10 20 30 40 50 60 70 80
 GGCAGCCAGCGGCCCTCAGACAGACCCACTGGCGCTCTCTGCTGAGTGACCGTAAGCTCGCGTCTGGCCCTCTGCCCTGC
 90 100 110 120 130 140 150 160
 CTCTCCCTGAGTGTGGCTGACAGCCACGCAGCTGTGTCTGTCTGCGGCCGTGCATCCCTGCTGCCGCCCTGGT
 170 180 190 200 210 220 230 240
 ACCTTCCTTGCCGTCTTCTCTGTCTGCTGCTCTGTGGGACACCTGCCCTGGAGGCCAGCTGCCGTCTCAGAGTG
 250 260 270 280 290 300 310 320
 ACAGGTCTTATGACAGCCTGATTGGTGAETGGGCTGGGTGTGGATTCTCACCCAGGCCCTGCCCTGCTTCTCAGACC
 330 340 350 360 370 380 390 400
 CTCATCTGTCACCCCACGCTGAACCCAGCTGCCACCCCCAGAACGCCATCAGACTGCCCTAGCACACGGAATGGATT
 410 420 430 440 450 460 470 480
 CTGAGAAAGGCCAACAGAAGGCCGTGGAGTCAGCATGCCCTGGCTGGCCGGGGCTTGCTCCTGCTGCTGC
 M P R G W A A P L L L L L L
 490 500 510 520 530 540 550 560
 TCCAGGGAGGCTGGGGCTGCCCTGACCTCGTCTGCTACACCGATTACCTCCAGACGGTCACTGCATCCGGAAATGGG
 O G G W G C P D L V C Y T D Y L O T V I C I L E M W
 570 580 590 600 610 620 630 640
 AACCTCCACCCAGCACGCTCACCTAACCTGGCAAGCCAGTATGAAGAGCTGAAGGACGAGGCCACCTCCTGCAGCC
 N L H P S T L T L T W Q D Q Y E E L K D E A T S C S L
 650 660 670 680 690 700 710 720
 CCACAGGTGGCCCACAATGCCACGCATGCCACCTACACCTGCCACATGGATGTATTCACCTCATGGCCACGACATT
 H R S A H N A T H A T Y T C H M D V F H F M A D D I F
 M P R M P P T P A T W H Y S T S W P T T F
 730 740 750 760 770 780 790 800
 TCAGTGTCAACATCACAGACCAGTCTGGCAACTACTCCAGGAGTGTGGCAGCTTCTCCTGGCTGAGAGCAAGTCCGAG
 S V N I T D Q S G N Y S Q E C G S F L L A E S K S E
 S V S T S Q T S L A T T P R S V A A F S W L R A S P R
 810 820 830 840 850 860 870 880
 GAGAAAGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCTCCCCCTGGAGTTCCGCAAAGACTCGAGCTATGAGCTGC
 E K A D L S G L K K C L P P P P P G V P Q R L E L *
 R K L I S V D S R S V S L L P L E F R K D S S Y E L O
 890 900 910 920 930 940 950 960
 AGGTGGGGCAGGGCCCATGCCCTGGCTCCTCCTACCAAGGGACCTGGAGTGAATGGAGTGAACCCGGTCACTTTCAGACC
 V R A G P M P G S S Y Q G T W S E W S D P V I F Q T

Figure 8

970 980 990 1000 1010 1020 1030 1040
 CAGTCAGAGGAGTTAAAGGAAGGCTGGAAACCCCTCACCTGCTGCTTCCTCCTGCCTGTCAAGTCCTCATTCCTGCCTT
 Q S E E L K E G W N P H L L L L L L L L V I V F I P A F
 1050 1060 1070 1080 1090 1100 1110 1120
 CTGGAGCCTGAAGACCCATCCATTGTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGC
 W S L K T H P L W R L W K K I W A V P S P E R F F M P
 1130 1140 1150 1160 1170 1180 1190 1200
 CCCTGTACAAGGGCTGCAGCGGAGACTTCAAGAAATGGGTGGGTGCACCCCTCACTGGCTCCAGCCTGGAGCTGGACCC
 L Y K G C S G D F K K W V G A P F T G S S L E L G P
 1210 1220 1230 1240 1250 1260 1270 1280
 TGGAGCCCAAGAGGTGCCCTCACCCCTGGAGGTGTACAGCTGCCACCCACCCAGCAGCCCTGTGGAGTGTGACTTCAACAG
 W S P E V P S T L E V Y S C H P P S S P V E C D F T S
 1290 1300 1310 1320 1330 1340 1350 1360
 CCCCCGGGGACGAAGGACCCCCCCCCGGAGCTACCTCCGCCAGTGGGTGGTCATTCCCTCCGCCACTTTCGAGCCCTGGACCC
 P G D E G P P R S Y L R Q W V V I P P P L S S P G P Q
 1370 1380 1390 1400 1410 1420 1430 1440
 AGGCCAGCTAATGAGGCTGACTGGATGTCCAGAGCTGGCCAGGCCACTGGCCCTGAGCCAGAGACAAGGTACCTGGGC
 A S * *
 1450 1460 1470 1480 1490 1500 1510 1520
 TGTGATGTGAAGACACCTGCAGCCTTGGTCTCTGGATGGCCTTGTGAGCCTGATGTTACAGTGTCTGTGTGTGATT
 1530 1540 1550 1560 1570 1580 1590 1600
 CATATGTGTGTGTGCATATGCATGTGTGTGTGTGTCTTAGGTGCCAGTGGCATGTCCACCGTGTGTGATT
 1610 1620 1630 1640 1650 1660 1670 1680
 GCACGTGCCTGTGGCCTGGATAATGCCCATGGTACTCCATGCATTCAACCTGCCGTGCATGTCTGGACTCACGGAGC
 1690 1700 1710 1720 1730 1740 1750 1760
 TCACCCATGTGCACAAGTGTGCACAGTAAACGTGTTGTGGTCAACAGAAAAAAAAAAAAAAAAAAAAAAA
 ?
 1770 1780
 AAAAAAAAAAAAAAAA

Figure 9

10 20 30 40 50 60 70 80
 GGCAGCCAGCGGCCTCAGACAGACCCACTGGCGTCTCTGCTGAGTGACCGTAAGCTGGCGTCTGGCCCTCTGCCCTGC
 90 100 110 120 130 140 150 160
 CTCTCCCTGAGTGTGGCTGACAGCCACGCAGCTGTCTGCTGCGGCCGTGCATCCCTGCTGCCGCCGCTGGT
 170 180 190 200 210 220 230 240
 ACCTTCCTGCCGCTCTCTTCCCTGTCTGCTCTGTGGGACACCTGCCTGGAGGCCAGCTGCCGTATCAGAGTG
 250 260 270 280 290 300 310 320
 ACAGGTCTTATGACAGCCTGATTGGTACTCGGCTGGGTGTGGATTCTCACCCCAAGGCCCTGCTGCTGCTTCAGACC
 330 340 350 360 370 380 390 400
 CTCATCTGTACCCCCAACGCTGAACCCAGCTGCCACCCCCAGAACGCCATCAGACTGCCCAAGCACACGGAAATGGATT
 410 420 430 440 450 460 470 480
 CTGAGAAAGAACGCCGAAACAGAACGGCCGTTGGGAGTCAGCATGCCCGTGGCTGGGCCGCCCTTGCTCCTGCTGC
 M P R G W A A P L L L L L
 490 500 510 520 530 540 550 560
 TCCAGGGAGGCTGGGCTGCCCGACCTCGCTGCTACACCGATTACCTCCAGACGGTCATCTGCATCCTGAAATGTGG
 Q G G W G C P D L V C Y T D Y L Q T V I C I L E M W
 570 580 590 600 610 620 630 640
 AACCTCCACCCCCAGCACGCTCACCCCTACCTGGCAAGACCAAGTATGAAGAGCTGAAGGACGAGGCCACCTCTGCAGCCT
 N L H P S T L T L T W Q D Q Y E E L K D E A T S C S L
 650 660 670 680 690 700 710 720
 CCACAGGTGGCCCACAATGCCACGATGCCACCTACACCTGCCACATGGATGTATTCCACTTCATGGCCGACGACATT
 H R S A H N A T H A T Y T C H M D V F H F M A D D I F
 730 740 750 760 770 780 790 800
 TCAGTGTCAACATCACAGACCAAGTCTGGCAACTACTCCCAGGAGTGTGGCAGCTTCTCCCTGGCTGAGAGCATCAAGCCG
 S V N I T D Q S G N Y S Q E C G S F L L A E S I K P
 810 820 830 840 850 860 870 880
 GCTCCCCCTTCAACGTGACTGTGACCTCTCAGGACAGTATAATATCTCCTGGCGTCAGATTACGAAGACCCCTGCCTT
 A P P F N V T V T F S G Q Y N I S W R S D Y E D P A F
 890 900 910 920 930 940 950 960
 CTACATGCTGAAGGGCAAGCTTCAGTATGAGCTGCAGTACAGGAACCGGGAGACCCCTGGCTGTGAGTCCGAGGAGAA
 Y M L K G K L Q Y E L Q Y R N R G D P W A V S P R R K
 970 980 990 1000 1010 1020 1030 1040
 AGCTGATCTCAGTGGACTCAAGAAGTGTCTCCCTCCTCCCCCTGGAGTCCGAAAGACTCGAGCTATGAGCTGCAGGTG
 L I S V D S R S V S L L P L E F R K D S S Y E L Q V
 1050 1060 1070 1080 1090 1100 1110 1120
 CGGGCAGGGGCCATGCCTGGCTCTCCCTACAGGGGACCTGGAGTGAATGGAGTGACCCGGTCATCTTCAGACCCAGTC
 R A G P M P G S S Y Q G T W S E W S D P V I F Q T Q S

Figure 10

1130 1140 1150 1160 1170 1180 1190 1200
 AGAGGAGTTAAAGGAAGGCTGGAACCCCTCACCTGCTGCTTCCTCCTGCCTGTGATAGTCCTCATTCCTGCCCTCTGGA
 E E L K E G W N P H L L L L L L L V I V F I P A F W S
 1210 1220 1230 1240 1250 1260 1270 1280
 GCCTGAAGACCCATCCATTGTGGAGGCTATGGAAGAAGATATGGGCCGTCCCCAGCCCTGAGCGGTTCTTCATGCCCTG
 L K T H P L W R L W K K I W A V P S P E R F F H P L
 1290 1300 1310 1320 1330 1340 1350 1360
 TACAAGGGCTGCAGCGGAGACTCAAGAAATGGGTGGGTGCACCCCTCACTGGCTCCAGCCTGGAGCTGGGACCCCTGGAG
 Y K G C S G D F K K W V G A P F T G S S L E L G P W S
 1370 1380 1390 1400 1410 1420 1430 1440
 CCCAGAGGTGCCCTCCACCCCTGGAGGTGTACAGCTGCCACCCACCGAGCCGCCAAGAGGCTGCAGCTCACGGAGC
 P E V P S T L E V Y S C H P P R S P A K R L Q L T E I
 1450 1460 1470 1480 1490 1500 1510 1520
 TACAAGAACCAAGCAGAGCTGGTGGAGTCTGACGGTGTGCCAAGCCAGCTCTGGCCGACAGCCAGAACACTCGGGGGC
Q E P A E L V E S D G V P K P S F W P T A Q N S G G
 1530 1540 1550 1560 1570 1580 1590 1600
 TCAGCTTACAGTGAGGAGAGGGATCGGCCATACGGCCTGGTGTCCATTGACACAGTGACTGTGCTAGATGCAGAGGGCC
S A Y S E E R D R P Y G L V S I D T V T V L D A E G P
 1610 1620 1630 1640 1650 1660 1670 1680
 ATGCACCTGGCCCTGCAGCTGTGAGGATGACGGCTACCCAGCCCTGGATGCTGGCTGGAGCCAGCCAGGCC
C T W P C S C E D D G Y P A L D L D A G L E P S P G L
 1690 1700 1710 1720 1730 1740 1750 1760
 TAGAGGACCCACTCTTGGATGCAGGGACCACAGTCCTGCTGTGGCTGTCTCAGCTGGCAGCCCTGGCTAGGAGGG
E D P L L D A G T T V L S C G C V S A G S P G I G G
 1770 1780 1790 1800 1810 1820 1830 1840
 CCCCTGGGAAGCCTCTGGACAGACTAAAGCCACCCCTGAGATGGGGAGGACTGGGCTGGGGACTGCCCTGGGTGG
P L G S L L D R L K P P L A D G E D W A G G L P W G G
 1850 1860 1870 1880 1890 1900 1910 1920
 CCGGTACCTGGAGGGCTCAGAGAGTGAGGCGGGCTACCCCTGGCCGGCCTGGATATGGACACGTTGACAGTGGCT
R S P G G V S E S E A G S P L A G L D M D T F D S G F
 1930 1940 1950 1960 1970 1980 1990 2000
 TTGTGGCTCTGACTGCAGCAGCCCTGTGGAGTGTGACTTCACCAAGCCCCGGGAGCAAGGGACCCCCCGGAGCTACCTC
V G S D C S S P V E C D F T S P G D E G P P R S Y L
 2010 2020 2030 2040 2050 2060 2070 2080
 CGCCAGTGGGTGGTCATTCCCTCCGCCACTTTCAGGCCCTGGACCCAGGCCAGCTAATGAGGCTGACTGGATGTCCAGAG
 R Q W V V I P P P L S S P G P Q A S * *

Figure 11

2090 2100 2110 2120 2130 2140 2150 2160
CTGGCCAGGCCACTGGGCCCTGAGCCAGAGACAAGGTACCTGGGCTGTGATGTGAAGACACCTGCAGCCTTGGTCTCC
2170 2180 2190 2200 2210 2220 2230 2240
TGGATGGGCCTTGTAGCCTGATGTTTACAGTGTCTGTGTGTGCATATGTGTGTGTGCATATGCATGTGTGTGTG
2250 2260 2270 2280 2290 2300 2310 2320
TGTGTGTCTTAGGTGCGCAGTGGCATGTCCACGTGTGTGATTGCACGTGCCTGTGGCCTGGATAATGCCCATGG
2330 2340 2350 2360 2370 2380 2390 2400
TACTCCATGCATTACCTGCCCTGTGCATGTCTGGACTCACGGAGCTACCCATGTGCACAAGTGTGCACAGTAAACGTG
2410 2420 2430 2440 2450 2460 2470 2480
TTTGTGGTCAACAGAaa

Figure 12

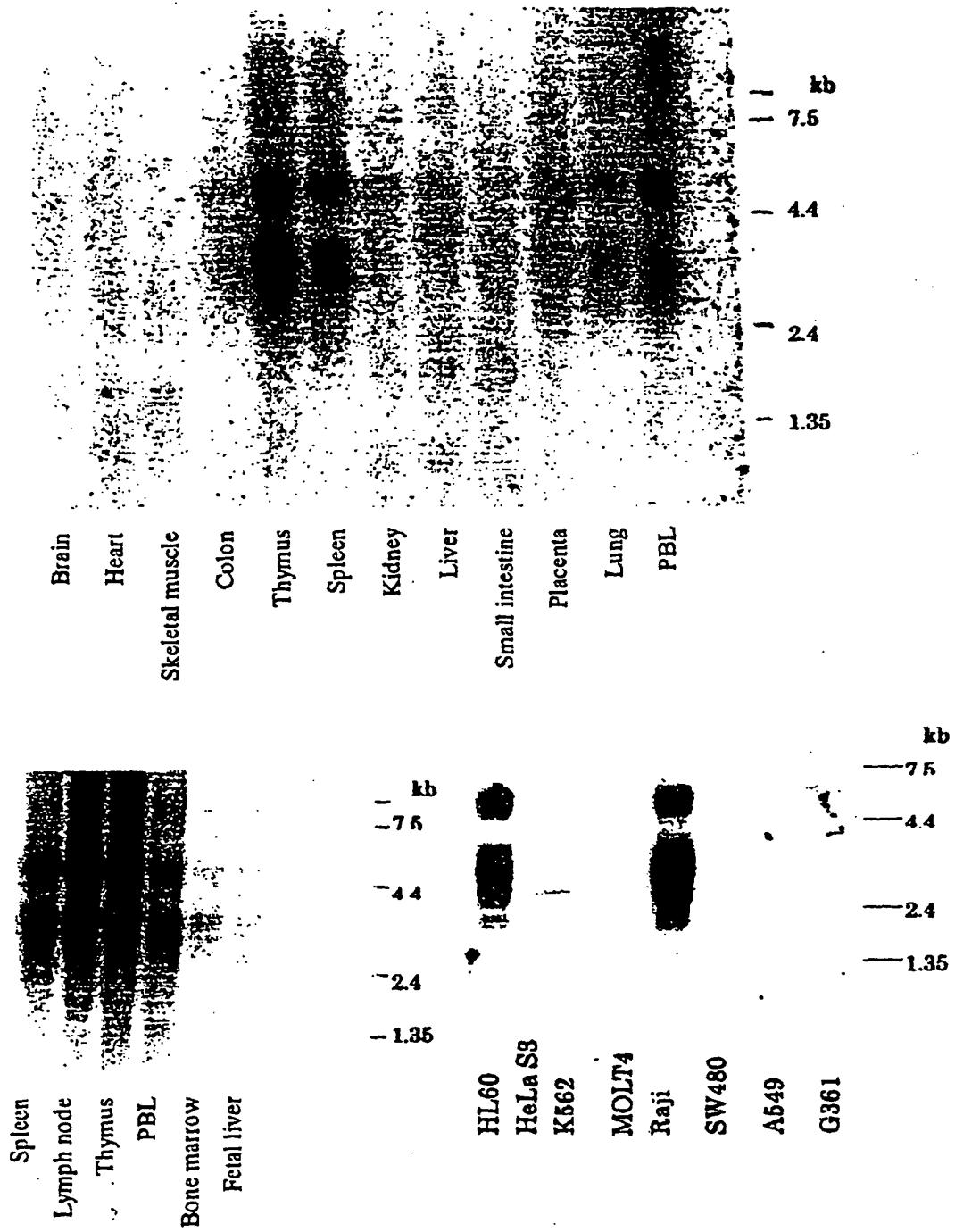


Figure 13

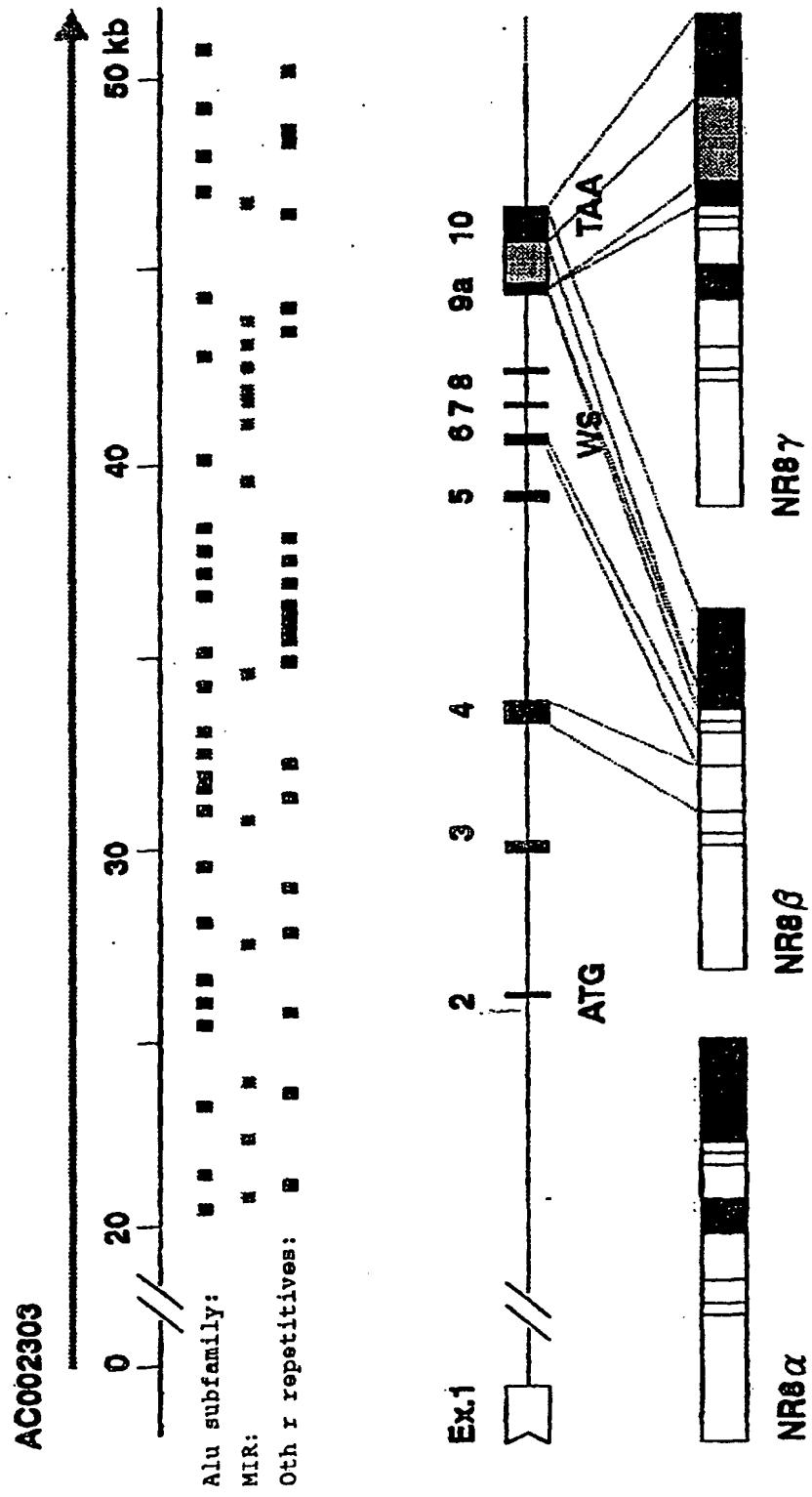
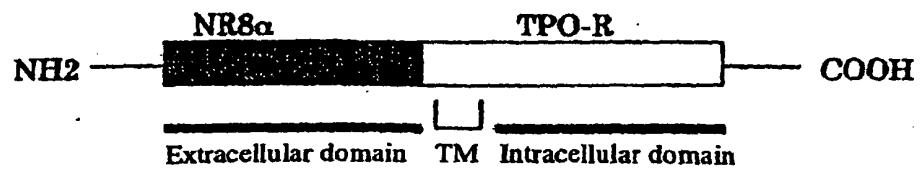


Figure 14

pEF-NR8/TPO-R



pEF-NR8/IgG-Fc



pEF-BOS/NR8b FLAG



Figure 15

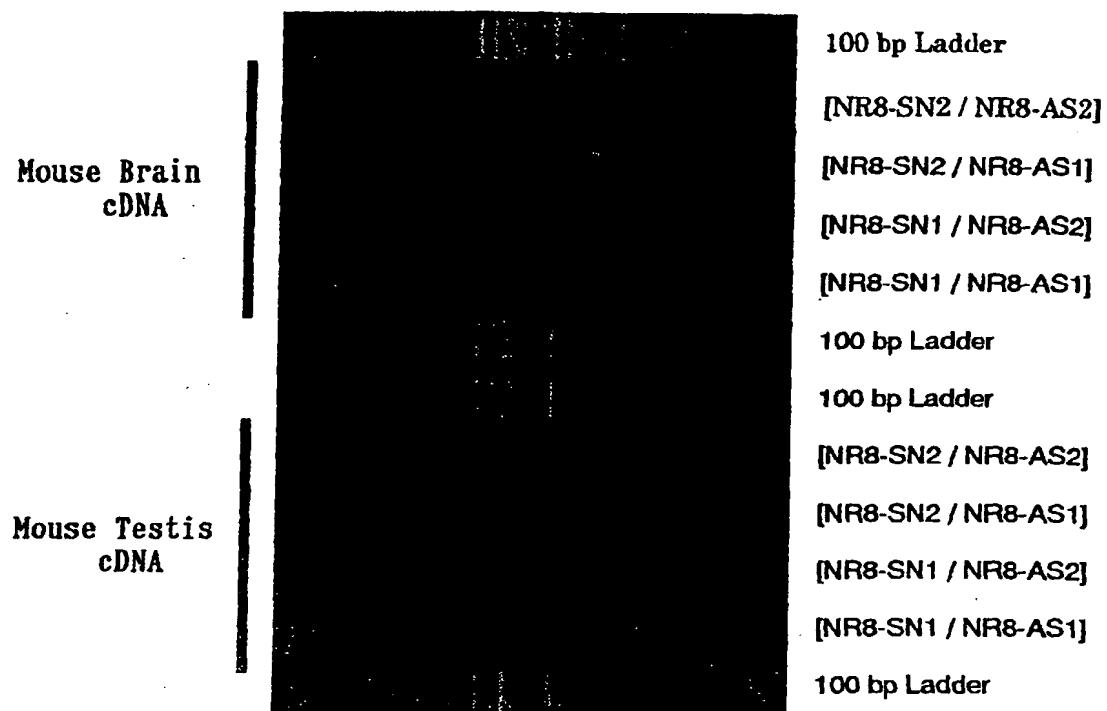


Figure 16

hNR8BETA	MERGWTAPLIV	KEEDEGSGWCG	PRDVYCEPDTG	QYNTICGEGWV	40
mNR8BETA	MEVGTAAASLG	KEEDEGSGWCG	PRDVYCEPDTG	QYNTICGEGWV	40
hNR8BETA	NEHRASGTYTEN	KGDDYVSEKGD	TAISGCSHRS	ABNATHRVEV	80
mNR8BETA	NEHPSSTETTEN	KGDDYVSEKGD	TAISGCSHRS	ABNATHRVEV	80
hNR8BETA	CBMIVYKHEMA	DLKPSVYVETD	DSGNIZSPPCG	SKLAESEKSI	120
mNR8BETA	SMIVYKHEMA	DLKPSVYVETD	DSGNIFDQG	SKPRAISXSI	120
hNR8BETA	EKADLISGLK	CMPPEPPGVHQ	RKPE		144
mNR8BETA	EKADLISGLK	CMPPEPPGVHQ	RKPE		144

Figure 17

hNR8G	MERGNAAPPL	LLIGECCGCGCC	PDIVGCTGCG	GTAGCTTAAW	40
mNR8G	MERGNAASPL	LLIGECCGCGCC	PDIVGCTGCG	GTAGCTTAAW	40
hNR8G	NEPESDIAATP	WQHDXEPLKE	EPNSGSHRS	ANATHACWY	80
mNR8G	NEPESDIAATP	WQHDXEPLKE	EPNSGSHRS	ANATHACWY	80
hNR8G	CHMDVCEHWA	DDIARSVNHTD	DSGNXSORCG	SELLAEECCE	120
mNR8G	SHMDVCEHWA	DDIARSVNHTD	DSGNXFORCE	SELLAEECCE	120
hNR8G	APPTNIVTATP	SGDQNTISWRIS	DYEDPAPLYME	KGKGQELION	160
mNR8G	APPTNIVTATP	SGDQNTISRHS	DYEDPAPLYME	KGKGQELION	160
hNR8G	AVRGDPWAVS	PRKIKLXVDS	RSVSKLPLRS	RKDSSEYLOV	200
mNR8G	RNGSDPWAVS	PRKIKLXVDS	RSVSKLPLRS	RKDSSEYLOV	200
hNR8G	KAGEMPESSY	QGINSMSDDE	WKGQDSEK	KKGNNRHEE	240
mNR8G	KAGEMPESSY	QGINSMSDDE	WKGQDSEK	KKGNNRHEE	240
hNR8G	EGGAVLWISIP	AVWSEKTHPI	QHIVKLSIMAV	PSPKKEEMEL	280
mNR8G	EGGAVLWISIP	AVWSEKTHPI	QHIVKLSIMAV	PSPKKEEMEL	280
hNR8G	YKGCGDPEKK	IVGAGETPGSS	LEIGENSPEV	PSLLEEVSCS	320
mNR8G	YKGCGDPEKK	IVGAGETPGSS	LEIGENSPEV	PSLLEEVSCS	320
hNR8G	PPRSPKAKHQ	ATTCGCGAEL	VESDIGVPIPS	EWPITQNSCG	360
mNR8G	PPRSPKAKHQ	ATTCGCGAEL	VESDIGVPIPS	EWPITQNSCG	360
hNR8G	SAYSEPRDHP	XGIVSITIVT	VLDAEGPCTW	PCSCEDDCTG	400
mNR8G	SAYSEPRDHP	XGIVSITIVT	VLDAEGPCTW	PCSCEDDCTG	400
hNR8G	ALIEDDAGLEP	SGCLERDDELD	ACTTATSCGG	VSAGSSEGECC	440
mNR8G	ALIEDDAGLEP	SGCLERDDELD	ACTTATSCGG	VSAGSSEGECC	440
hNR8G	PIGSLLDREK	PPRAGEDGWA	GGPAGGRSP	GGVSESEAGS	480
mNR8G	PIGSLLDREK	PPRAGEDGWA	GGPAGGRSP	GGVSESEAGS	480
hNR8G	PIAGLDMDTP	DSGPVGSDCS	SPVECDPTSP	GDEGPPRSYI	520
mNR8G	PIAGLDMDTP	DSGPVGSDCS	SPVECDPTSP	GDEGPPRSYI	520
hNR8G	ROWVVIPEPL	SSPGDQAS			538
mNR8G	ROWVVIPEPL	SSPGDQAS			538

Figure 18



100 bp Ladder

E17-day

E15-day

E11-day

E7-day

Testis

Kidney

Skeletal muscle

Liver

Lung

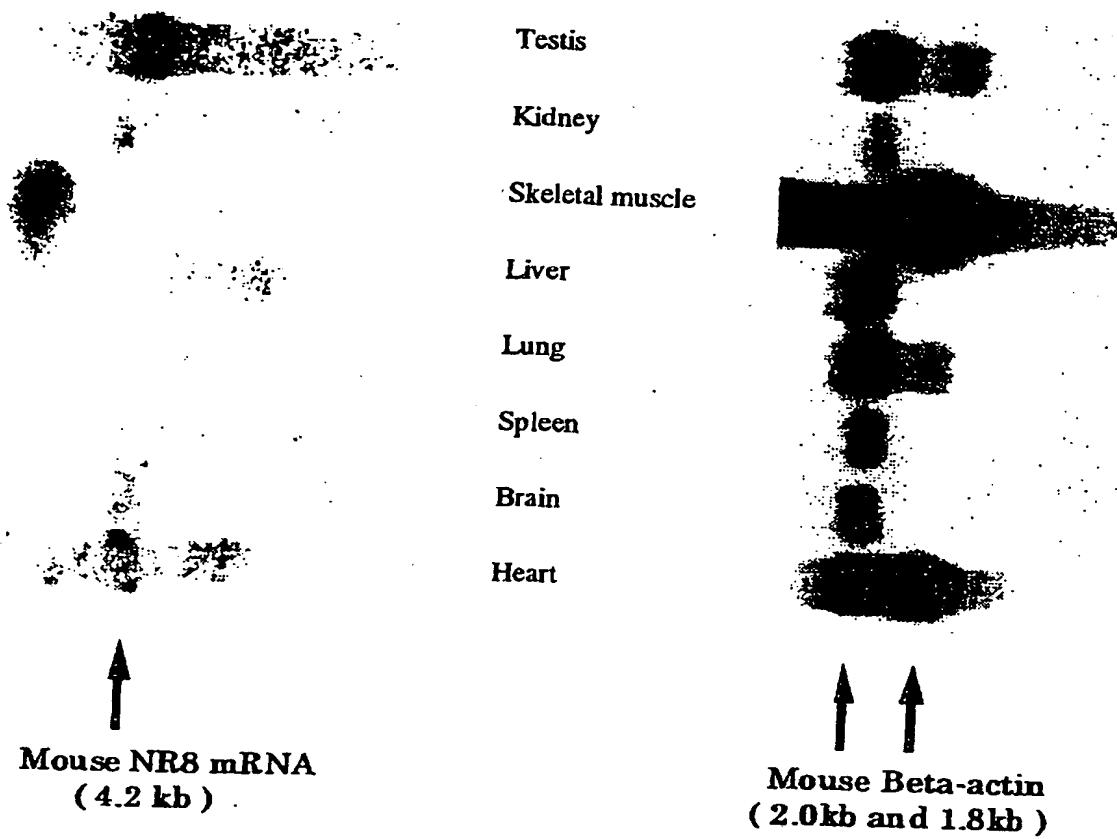
Spleen

Brain

Heart

100 bp Ladder

Figure 19



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP99/03351

A. CLASSIFICATION OF SUBJECT MATTER
 Int.Cl⁶ C07K14/715, C07K19/00, C12N15/12, C12N5/10, C12P21/02,
 G01N33/50, C07K16/16/28, G01N33/53, C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl⁶ C07K14/715, C07K19/00, C12N15/12, C12N5/10, C12P21/02,
 G01N33/50, C07K16/16/28, G01N33/53, C12Q1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 GenBank/EMBL/DDBJ/GeneSeq, SwissProt/PIR/GeneSeq**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Biol. Chem. 271[23] (1996) Robb L. et al., "Structural Analysis of the Gene Encoding the Murine Interleukin-11 Receptor α -Chain and a Related Locus" p.13754-13761	1-21
A	Proc. Natl. Acad. Sci. USA 93 (1996) Gainsford T. et al., "Leptin can induce proliferation, differentiation, and functional activation of hematopoietic cells" p.14564-14568	1-21
A	Proc. Natl. Acad. Sci. USA 93 (1996) Hilton D.J. et al., "Cloning and Characterization of a binding subunit of the interleukin 13 receptor that is also a component of the interleukin 4 receptor" p.497-501	1-21

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Date of the actual completion of the international search 24 September, 1999 (24. 09. 99)	Date of mailing of the international search report 5 October, 1999 (05. 10. 99)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer

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